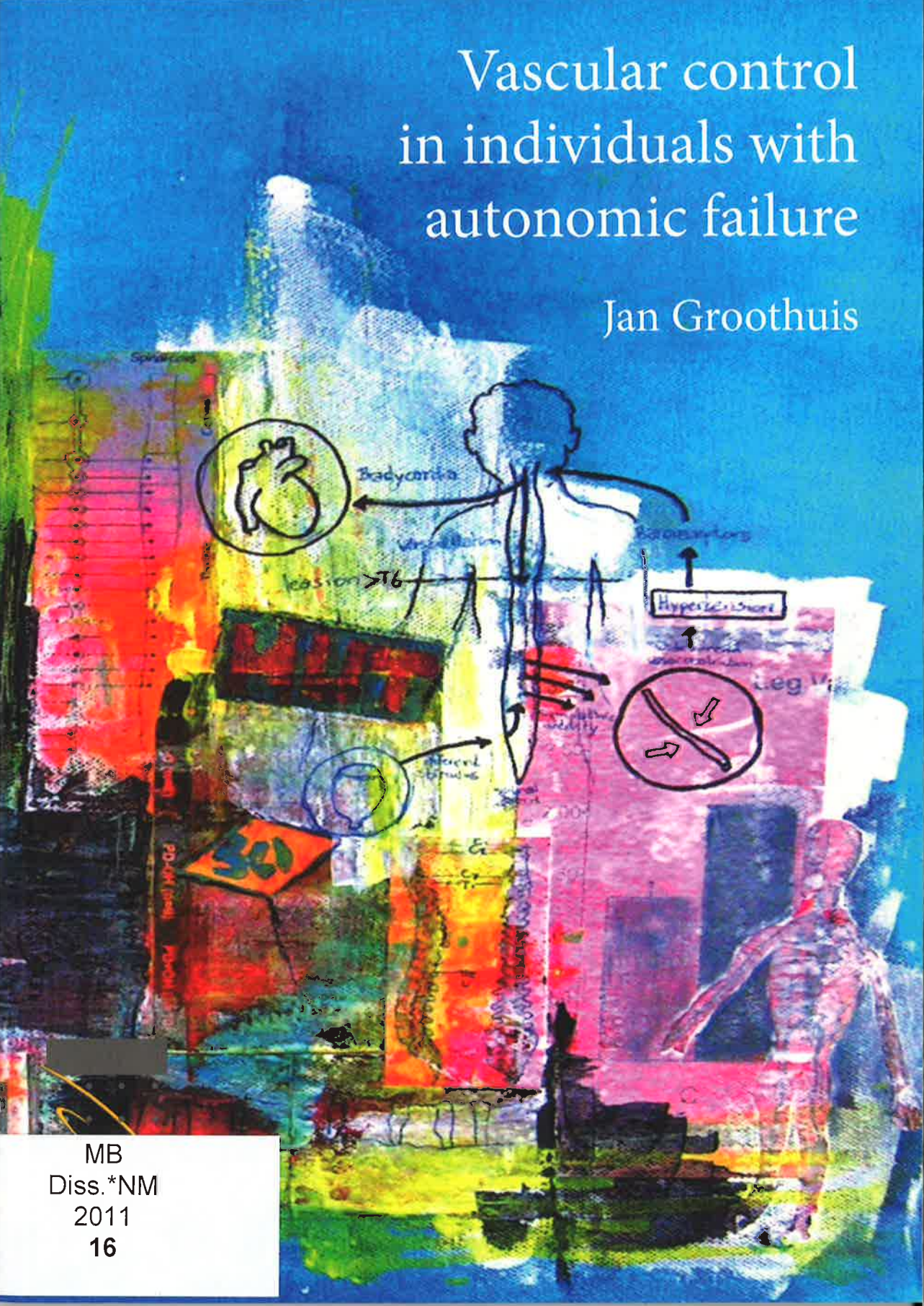
Vascular control in individuals with autonomic failure

Jan Groothuis



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Vascular control in individuals with autonomic failure

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CONTENTS

General introduction

1

1

About seven million years ago one of the most important moments of the human evolution occurred when a group of new beings, descendents of the chimpanzees, emerged from the tropical forests to the open savannas of Africa. These new beings had one major prevailing characteristic: they were capable of standing and walking upright. Over a period of millions of years the modern human being, *Homo sapiens*, evolved some two hundred thousand years ago. Over this period the modern human being evolved to a full erected posture when standing and walking, completing the evolutionary transition from quadruped to bipedal human being.

The upright posture enhanced mobility, but at the same time it greatly challenged the control of blood pressure regulation by making it highly sensitive to gravitational forces. The blood pressure regulation systems in most animals are quite similar with a large pump, the heart, circulating the blood to a large system of blood vessels. However, in most quadrupeds about 70% of blood volume is *at or above* heart level in contrast to humans where approximately 70% of blood volume is *below* heart level (1). This means that in quadrupeds the hydrostatic and gravitational impact on blood pressure regulation is significantly less than in humans. To maintain blood pressure in the upright posture, blood pressure regulation systems in humans evolved over time to accommodate for gravitational and hydrostatic forces (1).

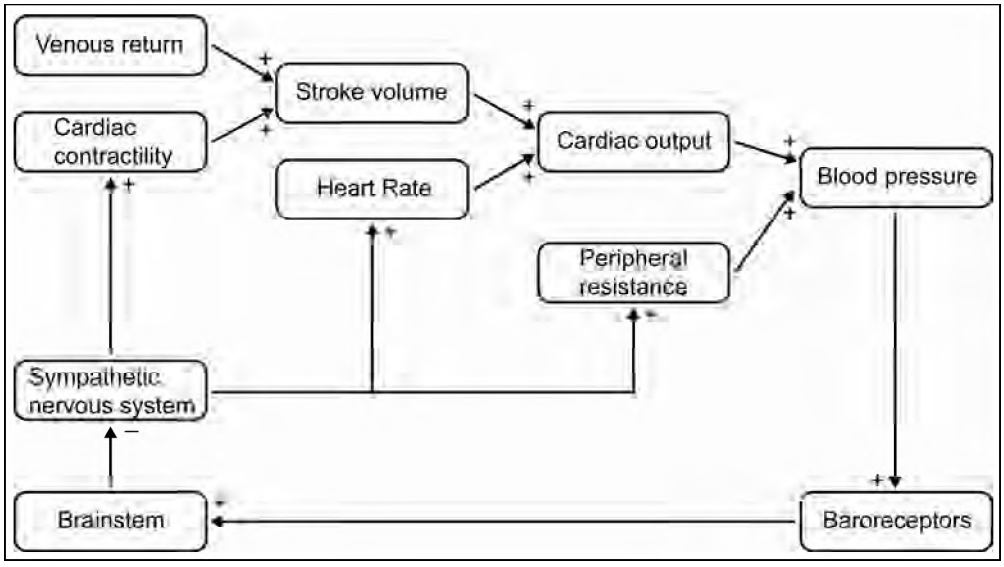
The autonomic nervous system, through sympathetic nervous system activity, plays a principal role in maintaining blood pressure during the upright position (2-4). It is, therefore, thought that an alteration of the function of the autonomic nervous system may result in a decrease in blood pressure when adopting the upright posture, known as orthostatic hypotension (2,4). The aim of this thesis is to examine the role of peripheral vascular control systems in individuals with autonomic failure. In this chapter the function of the autonomic nervous system in the blood pressure regulation will be summarised with special emphasis on the sympathetic nervous system mediated peripheral vascular control.

BLOOD PRESSURE REGULATION

Maintaining blood pressure during the upright posture is essential for our well being. It is socially not desirable or even not accepted that individuals cannot stand-up or stand upright for a certain amount of time. This is only possible when the blood pressure is maintained during the upright posture by regulating mechanisms. Blood

pressure is defined as the product of cardiac output (heart rate times stroke volume) and total peripheral resistance (Figure 1). The effect of the upright posture on cardiac output has been extensively studied. In contrast, the effect of the upright posture on peripheral vascular resistance is scarcely studied.

Figure 1. Schematic representation of baroreflex control of blood pressure through changes in sympathetic nervous system activity.



Vasoconstrictor mechanisms

The upright posture in humans results in a gravitational displacement of ~500-1000 ml of blood into the dependent vasculature below the diaphragm, leading to a decrease in venous return to the heart with a consequent decrease in cardiac output and an immediate drop in blood pressure (Figure 1) (3,4). The autonomic nervous system plays a principal role in the blood pressure regulation and the maintenance of blood pressure during changes in posture (2-4). The blood pressure drop during the upright posture will unload the baroreceptors resulting in an increased sympathetic nerve activity and decreased vagal outflow, i.e. baroreflex activation (2). The baroreceptors are stretch receptors located in the carotid sinus and ascending aorta. In order to maintain blood pressure, the baroreceptor mediated activation of the sympathetic nervous system will increase heart rate, cardiac contractility and peripheral vascular resistance (Figure 1) (2,3). The primary role of the baroreflex is a

rapid adjustment of blood pressure on a beat-to-beat basis. Changes in peripheral vascular resistance rather than changes in cardiac output are thought to be primarily responsible for maintaining blood pressure during orthostatic challenges (3,5,6).

Although the baroreflex mediated increase in peripheral resistance is important in the maintenance of blood pressure during orthostatic challenges, there are also other mechanisms which can increase peripheral vascular resistance (4). During the initial adjustments to the change in posture, local vasoconstrictor mechanisms can contribute to the increased peripheral vascular resistance. These local vasoconstrictor mechanisms are the veno-arteriolar axon reflex (VAR) (7) and the myogenic response (8,9). It is thought that they may contribute to 45% of leg vasoconstriction during orthostatic challenges (7). Both are triggered by an increase in pressure caused by the gravitational displacement of blood during the upright posture. The VAR is triggered by an increase in venous pressure resulting in a vasoconstriction of the corresponding arteriole (7). Whereas the myogenic response is triggered by an increase in transmural pressure across an arteriole (8,9). During prolonged orthostatic stress, the baroreflex mediated increase in sympathetic activity remains important (4), but also humoral factors, such as angiotensin II, contribute to the increased peripheral resistance (4,10). The renin-angiotensin-aldosterone system is activated during orthostatic challenges via increased sympathetic activity to the kidneys and renal hypoperfusion. The released renin will catalyse the conversion of angiotensinogen to angiotensin I, which will be converted to angiotensin II, via the angiotensin converting enzyme (ACE) (11,12). Angiotensin II can induce vasoconstriction via binding to angiotensin II type 1 receptors (11,12). Vasopressin and atrial natriuretic factor seem to play only a minor role in the maintenance of blood pressure during orthostatic challenges (4,10). Also the local endothelium derived vasoconstrictor endothelin-1 does not seem to play a role in maintaining blood pressure during orthostatic challenges (13).

Orthostatic hypotension

Failure of these regulating mechanisms to maintain blood pressure during the upright position can result in (a)symptomatic orthostatic hypotension (2,4,14-16). This is defined as a decrease in systolic blood pressure of at least 20 mmHg or a decrease in diastolic blood pressure of at least 10 mmHg within 3 minutes of standing or a 60° head-up tilt test (17). Orthostatic hypotension is often the most disabling symptom in patients and negatively related to daily life activities and quality of life (4,18-22). It

increases the risk of falls (23-25) and is associated with cardiovascular morbidity and mortality in elderly (26-30). Even in the general population it is estimated that orthostatic hypotension occurs in 0.5% (31), with a higher incidence of 5-30% in the elderly (29,32-34). Characteristic symptoms of orthostatic hypotension are mostly due to cerebral hypoperfusion and include feelings of weakness, fatigue, blurred vision, light headedness, dizziness and loss of consciousness (2,17,19).

AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system, through sympathetic nervous system activity, plays a principal role in maintaining blood pressure during the upright position (2-4). Orthostatic hypotension in patients with autonomic disorders is thought to be related to baroreflex failure (4,14-16). A normal function of the autonomic nervous system and particularly the sympathetic mediated increase in peripheral vascular resistance is, therefore, essential to maintain blood pressure. In this chapter we will focus on the sympathetic nervous system regulation of blood pressure.

Anatomy and physiology

The autonomic nervous system has a central and peripheral part. The central part exists of multiple brain areas, such as the hypothalamus and 'limbic system', including the hippocampus, amygdale and insula cortex. Within the brainstem the input and output of the central and peripheral autonomic nervous system are integrated to control vasomotor tone and cardiac function from the ventrolateral medulla in the brainstem. The peripheral autonomic nervous system consists of two complementary system, e.g. the sympathetic and parasympathetic nervous system. The sympathetic nervous system is also known as the 'fight and flight' system, whereas the parasympathetic nervous system is known as the 'rest and digest' system.

The sympathetic nervous system exists of pre- and postganglionic fibres (Figures 2 and 3). The preganglionic axons derive from cell bodies located within the spinal cord between the first thoracic (T1) and the second or third lumbar spinal segment (L2-3). These efferent preganglionic fibres innervate sympathetic neurons located in para- and prevertebral ganglia. Efferent postganglionic axons from these ganglia innervate the target organs. The paravertebral ganglia are paired at both sides of the vertebral column forming the sympathetic trunk. The heart is innervated from

the preganglionic axons derived from the first to fourth thoracic spinal segment (T1-4). Smooth muscle cells of peripheral blood vessels are innervated from their corresponding spinal cord segments, for example blood vessels of the trunk at thoracic segments and of the lower limbs from low thoracic and lumbar spinal segments. Preganglionic axons innervating the splanchnic organs pass through the sympathetic trunk to prevertebral ganglia and derive from spinal segments below the sixth thoracic spinal segment (T6).

Figure 2. The sympathetic autonomic nervous system with outflow to various organs.

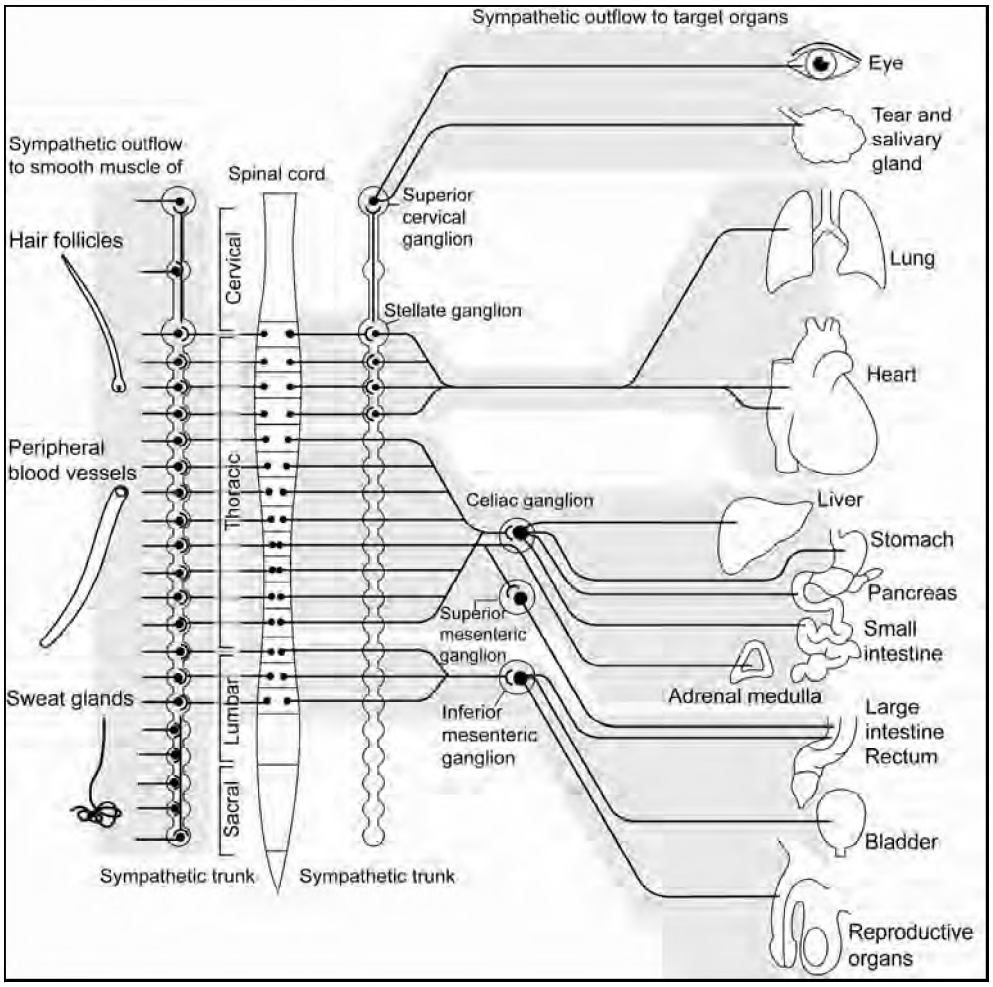


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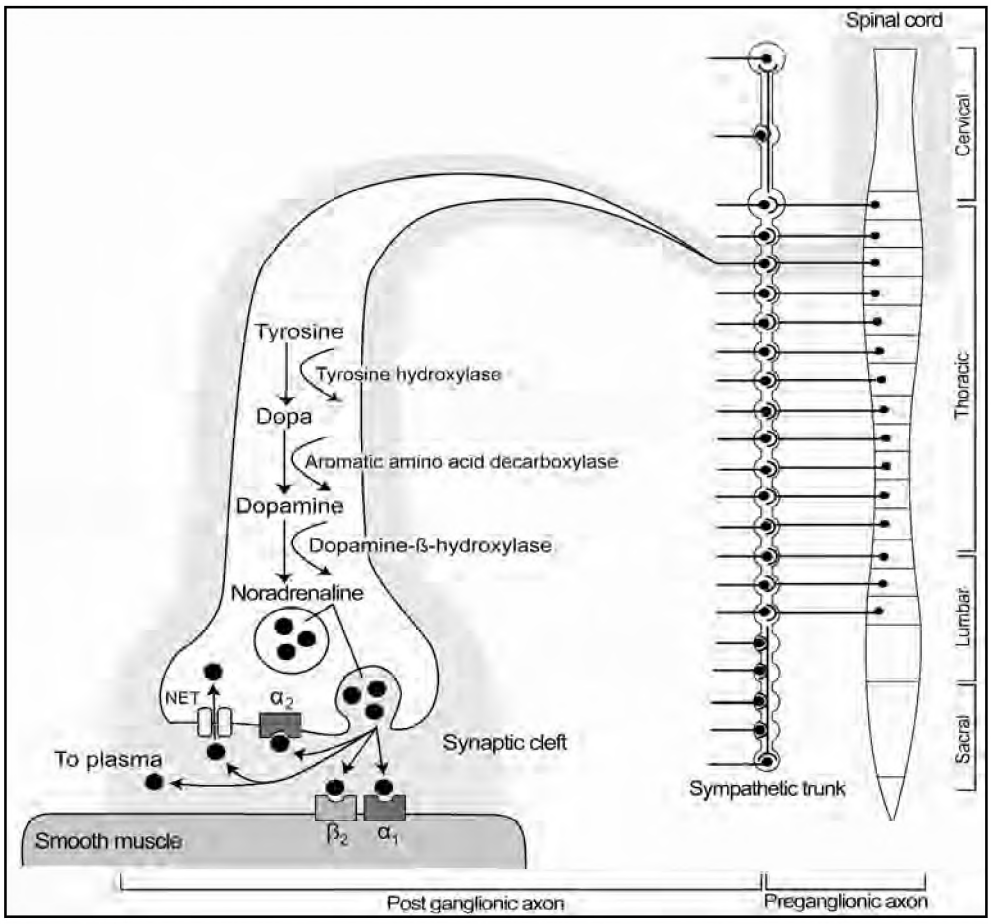
Figure 3. The sympathetic nervous system.



Via pre- and postganglionic axons, an electrical potential releases noradrenaline (●) from the nerve terminal into the synaptic cleft where it binds to adrenoceptors (α_1 and $ß_2$) of the smooth muscle cell of the target organ. The presynaptic α_2-adrenoceptor will inhibit noradrenaline release and the noradrenaline transporter (NET) will reuptake noradrenaline from the synaptic cleft.

In the sympathetic nervous system, noradrenaline is the effective neurotransmitter released from the postganglionic nerve terminals, whereas acetylcholine is the neurotransmitter at the preganglionic nerve terminals, with exception of the sweat glands where acetylcholine is the effective neurotransmitter. The biosynthesis of noradrenaline occurs in the sympathetic neuron and starts with the amino acid tyrosine which is conversed into dihydroxyphenylalanine (DOPA) catalysed by tyrosine hydroxylase (Figure 3). In the second step, L-aromatic amino acid decarboxylase catalyses the conversion of DOPA to dopamine. Finally, dopamine is

conversed into noradrenaline which is catalysed by dopamine-ß-hydroxylase. Noradrenaline will be released from the nerve terminal into the synaptic cleft triggered by electrical action potentials. Binding of noradrenaline to the α- and ß-adrenergic receptors of target organs will cause the major sympathetic actions (Figure 3).

Besides noradrenaline also non-adrenergic neurotransmitters, such as adenosine triphosphate (ATP) and neuropeptide Y (NPY), are released from the sympathetic nerve terminal (36,37). These neurotransmitters are co-stored with noradrenaline, simultaneously released into the synaptic cleft and thought to have a coordinated action with noradrenaline (36,37). ATP acts on the purinoceptor P2X of the target organ and has been demonstrated to be a vasoconstrictor in animal studies (36). Neuropeptide Y modulates the action of noradrenaline and ATP, and does not seem to be a vasoconstrictor itself (36).

Autonomic disorders

There are many different disorders which can result in autonomic failure (Table 1). We distinguish primary autonomic disorders, with unknown aetiology, from secondary disorders (38). The primary autonomic disorders are subdivided in acute and chronic autonomic failure syndromes. Pure autonomic failure, multiple system atrophy, formerly known as the Shy-Drager syndrome, and autonomic failure with Parkinson's disease are classified as primary chronic autonomic disorders. Secondary autonomic disorders cover a wide range of causes from common disorders, such as diabetes mellitus, via quite specific disorders, such as spinal cord injuries, to very rare hereditary disorders, such as dopamine-ß-hydroxylase deficiency. There are also various drugs, chemicals, poisons and toxins which can cause autonomic failure. Besides these generalised autonomic disorders, there are also some localised autonomic disorders of which the most familiar is the Horner's syndrome. The autonomic disorders studied in this thesis will be discussed in more detail.

Pure autonomic failure

The first cases of pure autonomic failure (PAF) were described by Bradbury and Eggleston in 1925 as 'idiopathic orthostatic hypotension' (39). In PAF there is a postganglionic lesion of the sympathetic nervous system with a generalised los of sympathetic fibres in the body and consequently reduced supine plasma

noradrenaline levels (Table 2) (16,40,41). PAF is one of the chronic primary autonomic disorders (Table 1) and characterised by persistent orthostatic hypotension in the absence of signs of central nervous system disease or other known causes of orthostatic hypotension (17,38,39). Besides orthostatic hypotension also other symptoms of widespread autonomic failure are usually present. The cause of PAF is still unknown, but it seems not to be inherited nor infectious (42). It occurs in middle-aged or elderly individuals with no preference for sex or race (15,42). PAF is a chronic and disabling disorder, but is thought not to be lethal.(15,42)

Table 1. Primary and secondary autonomic disorders.

Primary autonomic disorders	**Secondary autonomic disorders**
Acute/subacute dysautonomias	*Congenital*
Pure cholinergic dysautonomia	Nerve growth factor deficiency
Pure pandysautonomia	*Hereditary*
Pandysautonomia with neurological features	Familial amyloid neuropathy
	Dopamine-ß-hydroxylase deficiency
Chronic autonomic failure syndromes	Familial dysautonomia
Pure autonomic failure	*Metabolic diseases*
Multiple system atrophy	Diabetes mellitus
Autonomic failure with Parkinson's disease	Chronic renal failure
Dementia with Lewy bodies	Chronic liver disease
	Alcohol induced
	Vitamin B_{12} deficiency
	Inflammatory
	Guillain-Barré syndrome
	Transverse myelitis
	Infections
	Bacterial: Tetanus
	Parasitic: Chagas'disease
	Viral: HIV
	Neoplasia
	Brain tumors
	Paraneoplastic
	Connective tissue disorders
	Rheumatoid arthritis
	Surgery
	Sympathectomy
	Trauma
	Spinal cord injury
	Drugs, chemicals, poisons and toxins

For the secondary autonomic disorders only the subdivisions and some examples are given.

Table 2. Pathophysiological classification of the different autonomic disorders

	PAF	PD	DBH	SCI
SNS lesion	Postganglionic	Postganglionic	Postganglionic	Central
Sympathetic nerves	Degenerative	Degenerative	Intact	Intact
Baseline noradrenaline	Low	Low	Absent	Normal
Noradrenaline release	Low	Low	Absent	Low
Response to noradrenaline	Increased	Increased	Increased	Increased

Spinal cord injury (SCI), Parkinson's disease with autonomic failure (PD), pure autonomic failure (PAF) and dopamine-ß-hydroxylase deficiency (DBH). SNS, sympathetic nervous system.

Parkinson's disease

James Parkinson, in 1817, accurately described the motor problems of what was later called Parkinson's disease (PD), but also noted several non-motor symptoms (43). PD is a neurodegenerative disorder caused by the loss of dopaminergic neurons in the substantia nigra and characterised by typical motor symptoms, i.e. bradykinesia or even akinesia, tremor and muscle rigidity (44). In the last decade it has become clear that PD also affects the sympathetic nervous system. Lewy bodies were found within the sympathetic ganglia (45,46) and there is sympathetic denervation of the heart (47,48), indicating a more generalised involvement (Table 2). Besides the typical motor symptoms also non-motor symptoms may be present, such as autonomic failure (47,49-53). Symptomatic and asymptomatic orthostatic hypotension occurs in up to 60% of PD patients (54-56) and was thought to be caused by levodopa treatment (57,58). However, PD patients off levodopa treatment or never treated with levodopa also demonstrate orthostatic hypotension (47,58,59). Orthostatic hypotension in PD, therefore, reflects autonomic failure and PD is now classified as a chronic primary autonomic disorder (42,47). PD usually occurs in older individuals with no preference for sex or race.

Dopamine-ß-hydroxylase deficiency

In the mid-eighties of the last century, the first two reported cases of dopamine-ß-hydroxylase (DBH) deficiency were published almost simultaneously by Robertson *et al.* (60) and Man in't Veld *et al.* (61). DBH deficiency is an extremely rare (less than 20 cases worldwide) autosomal recessive hereditary disorder (Table 1) (20). DBH deficiency is characterised by a complete absence of noradrenaline with an intact sympathetic nervous system (Table 2) (20). Since these patients lack the DBH enzyme,

they cannot convert dopamine into noradrenaline (20,60,61). As a result, noradrenaline levels are undetectable and dopamine levels are high (20,60-62). Autonomic failure in these patients is caused by a lack of sympathetic neurotransmitter, and not by a decreased or absent sympathetic activity, since the sympathetic nerves are intact (20). DBH deficiency patients suffer from severe orthostatic hypotension (20,60,61). DBH deficiency patients can be treated with L-threo-dihydroxyphenylserine (L-DOPS or droxidopa) (20,63), a synthetic prodrug of noradrenaline (64).

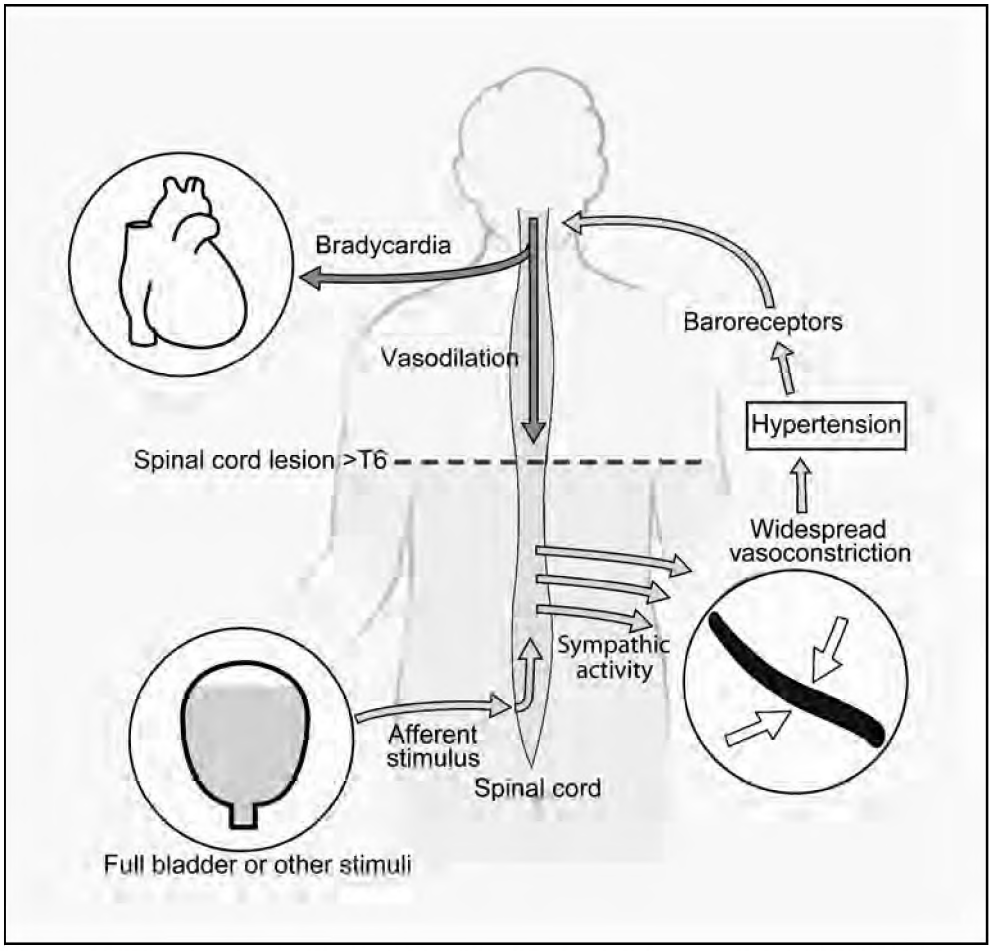
Spinal cord injury

The incidence of spinal cord injuries in the Netherlands varies between 10-27 per million individuals (65,66), mostly caused by trauma (67). Disruption of the spinal cord results in a complete or incomplete paralysis of motor, sensory and autonomic function below the level of the lesion. A spinal cord injury has dramatic consequences on the cardiovascular control by the sympathetic nervous system (68-70). The severity of the autonomic failure is depending on the completeness and level of the spinal cord lesion (68,69,71). At the level of the spinal cord lesion sympathetic denervation might take place, although sprouting of nerve cells might result in preservation of sympathetic nerves (72-74). Below the spinal cord lesion sympathetic nerves are preserved (69), however they are without central supraspinal control, depending the completeness of the lesion. Orthostatic hypotension is frequently demonstrated directly after the spinal cord lesion and persists over time in cervical and high thoracic spinal cord lesions (68-70,75). In lower thoracic spinal cord lesions orthostatic hypotension might resolve or become less important.

One of the cardiovascular consequences of a spinal cord injury is the increased basal leg vascular resistance (76), which might contribute to the development of pressure sores and poor wound healing in SCI individuals due to decreased perfusion (77). Previous research of our department has demonstrated that the increased basal leg vascular resistance in SCI individuals is not explained by adaptations in α-adrenergic vascular tone (78) or in endothelium derived nitric oxide (79). Endothelin-1 does contribute to the increased basal leg vascular resistance, however it cannot fully explain the total increase in leg vascular resistance in SCI individuals (80). Other vasoconstrictors might, therefore, contribute to the increased basal leg vascular resistance. Of special interest is angiotensin II, which is closely linked with

endothelin-1 (81-83) and SCI individuals have an increased renin-angiotensin II system activity (84,85). However, the role of angiotensin II in the increased basal leg vascular resistance in SCI individuals is unknown.

Figure 4. Schematic representation of autonomic dysreflexia in a spinal cord-injured individual with a spinal cord lesion above the sixth thoracic spinal segment (T6).



Modified with permission from the Canadian Medical Association from Blackmer, CMAJ 2003 (87).

Because of the intact sympathetic nervous system below the spinal cord lesion with no influence of supraspinal inhibitory pathways (69), the interesting clinical phenomenon of autonomic dysreflexia can occur in spinal cord-injured (SCI) individuals. Autonomic dysreflexia is a potentially life-threatening episode of hypertension that can develop in up to 80% of SCI individuals with a spinal cord

lesion at or above the sixth thoracic spinal segment (T6) (68). This phenomenon occurs in SCI individuals since a large part of the sympathetic nervous system has no central inhibitory pathways to limit the increase in blood pressure (69,70). Autonomic dysreflexia is characterised by a blood pressure increase, with levels as high as 300 mmHg systolic and 200 mmHg diastolic (68,69,71). The increase in blood pressure is induced by exaggerated sympathetic activity caused by visceral, noxious or nociceptive stimuli entering the spinal cord below the level of the lesion (Figure 4) (68-70,86,87). Daily trivialities in SCI individuals such as catheterization, bladder distension, and bowel evacuation can initiate autonomic dysreflexia (68-70,86,87). The increased blood pressure will result in a vasodilator response above the spinal cord lesion through loading of the baroreflex (Figure 4) (68-70,86,87). This results in the accompanying symptoms such as sweating, flushing and a pounding headache (68,86). Autonomic dysreflexia can lead to severe morbidity and even mortality in SCI individuals (88-91). Clinically, autonomic dysreflexia is well documented, but the mechanisms that mediate autonomic dysreflexia remain unclear (92). Because autonomic dysreflexia is induced by exaggerated sympathetic activity, it is thought to be α-adrenergic mediated (69,70,86).

The management of autonomic dysreflexia exist of non-pharmacological and pharmacological measures. The non-pharmacological measures are removing the trigger evoking autonomic dysreflexia and placing the SCI individual in a more upright position (86,93). Several pharmacological agents, mostly anti-hypertensives, have been used in an attempt to manage autonomic dysreflexia (93). In earlier days, α-adrenoceptor antagonists were used (86) and are a rational first choice in autonomic dysreflexia management considering the supposed mechanism. Nowadays, nifedipine, a calcium channel blocker, is most commonly used (86,93). Also the use of an angiotensin-converting enzyme (ACE) inhibitor, captopril, has been proposed as the primary pharmacological agent in the management of autonomic dysreflexia (94). The management of autonomic dysreflexia in clinical practice, therefore, remains a challenge (87,93).

OUTLINE OF THE THESIS

Aim of the present studies

Maintaining blood pressure during changes in posture has been an important human evolutionary achievement. The Autonomic nervous system plays a principal role in the control of blood pressure regulation via changes in sympathetic nervous system activity. Baroreflex mediated increases in sympathetic nervous system activity lead to changes in heart rate, cardiac contractility and peripheral vascular resistance. Surprisingly, the changes in peripheral vascular resistance are scarcely studied. Therefore, the general aim of this thesis is to examine the role of the peripheral vascular control in individuals with autonomic failure, with a special interest in individuals with a spinal cord lesion.

Head-up tilt is a frequently used test to establish orthostatic hypotension and to diagnose autonomic failure. To calculate peripheral vascular resistance during head-up tilt it is necessary to assess the changes in the arterial-venous pressure gradient. We, therefore, measured intravenous pressure during head-up tilt in *Chapter 2*. We hypothesize that leg venous pressure is similar to the calculated hydrostatic pressure and, therefore, that hydrostatic pressure makes an equal contribution to leg arterial as well as to leg venous pressure during head-up tilt.

SCI individuals with a complete spinal cord transsection have lost supraspinal control of the sympathetic nervous system below the spinal cord lesion. In *Chapter 3* we assess the leg vascular resistance changes in SCI individuals during head-up tilt. We hypothesize that SCI individuals have an attenuated leg vasoconstriction during head-up tilt caused by the loss of supraspinal sympathetic control.

Angiotensin II might contribute to the increased basal leg vascular resistance in SCI individuals. Moreover, during head-up tilt renin levels rise more quickly and to higher levels in SCI individuals than in controls, suggesting a role for angiotensin II in peripheral vasoconstriction during head-up tilt. Therefore, the role of angiotensin II in the increased leg vascular resistance in SCI individuals is assessed in *Chapter 4*. We hypothesize that angiotensin II contributes to the increased leg vascular resistance as well as to the leg vasoconstriction during head-up tilt in SCI individuals.

The clinical phenomenon of autonomic dysreflexia in SCI individuals is induced by exaggerated sympathetic activity and thought to be α-adrenergic mediated. In *Chapter 5* the α-adrenergic contribution to the increased leg vascular resistance during autonomic dysreflexia in SCI individuals is assessed. We hypothesize that α-adrenergic blockade during autonomic dysreflexia abolishes the increase in leg vascular resistance.

In *Chapter 6* we assess the leg vasoconstriction during head-up tilt in older men. An important characteristic in older men is the chronically elevated sympathetic nervous system activity. However, total peripheral vascular resistance is augmented in older men during head-up tilt. We hypothesize that leg vasoconstriction is also augmented in older men during head-up tilt, despite their already chronically elevated sympathetic activity.

Parkinson's disease is a primary autonomic disorder with pre- and postganglionic autonomic failure. In contrast to SCI individuals, it is assumed that Parkinson's disease patients have sympathetic denervation. In *Chapter 7* we assess the leg vascular resistance changes during head-up tilt in Parkinson's disease patients with and without orthostatic hypotension. We hypothesize that leg vasoconstriction during head-up tilt will be attenuated in Parkinson's disease patients with orthostatic hypotension.

Autonomic failure can be caused by lesions in different parts of the sympathetic nervous system, depending on the pathophysiology of the autonomic disorder. In *Chapter 8,* leg vascular responses during head-up tilt are assessed in two distinctive autonomic disorders with postganglionic autonomic failure, i.e. pure autonomic failure and dopamine-ß-hydroxylase deficiency. We hypothesize that leg vasoconstriction during head-up tilt will be present in pure autonomic failure and dopamine-ß-hydroxylase deficient patients despite their loss of sympathetic mediated vascular control, because of compensatory vasoconstrictor mechanisms.

Methods applied in this thesis

Blood pressure

Blood pressure is measured continuously using a non-invasive blood pressure device (Portapres, TNO, The Netherlands; Nexfin, BMEYE, The Netherlands) with an infrared finger photoplethysmograph. A finger cuff is attached to the middle phalanx of the right third finger in order to measure finger arterial blood pressure, which accurately reflects intra-arterial blood pressure changes (95). A built-in heart reference system was in operation to correct for hydrostatic influences. Mean arterial blood pressure is derived beat-to-beat and heart rate is the inverse of the interbeat interval. Modelflow, a pulse-contour method (96) is used to calculate stroke volume (97), cardiac output and total or systemic peripheral resistance (98).

Venous occlusion plethysmography

Leg resistance arterial blood flow is measured by electrocardiography-triggered venous occlusion plethysmography (99), using electrically calibrated mercury-in-silastic strain gauges (100). The leg is positioned approximately 5 cm above heart level to facilitate venous outflow between venous occlusions (101). For upper leg (thigh) blood flow measurements the strain gauge is placed around the upper leg, 10 cm above the patella. A venous occlusion cuff, placed around the upper leg 5 cm above the strain gauge, is rapidly inflated with a rapid cuff inflator (Hokanson E-20, Hokanson, USA), within 1 second, to 50 mmHg (102). Occlusion pressures are sustained for 8 heart cycles after which the cuff was deflated instantaneously for 10 heart cycles. Blood flow is calculated as the slope of the volume change over a 4 seconds interval using a customized computer program (103). Measurement of upper leg blood flow using venous occlusion plethysmography has a coefficient of variation between 6 and 9% in our laboratory (103).

Similarly, lower leg (calf) blood flow can be measured using venous occlusion plethysmography (99). The strain gauge is placed around the thickest part of the calf and a venous occlusion cuff is placed around the distal part of the upper leg. Measurement of calf blood flow using venous occlusion plethysmography has a coefficient of variation between 13 and 14% in our laboratory (103).

We used venous occlusion plethysmography in the supine and head-up tilt position. During head-up tilt, the leg blood flow measurement with venous occlusion

plethysmography could be influenced by venous compliance. Venous occlusion plethysmography is assumed to require an empty venous system to guarantee full venous compliance (99,101). However, leg blood flow measured using venous occlusion plethysmography during head-up tilt is in good agreement with superficial femoral artery blood flow measured by Doppler ultrasound (correlation coefficient of 0.86) (104). This indicates that venous occlusion plethysmography during head-up tilt represents arterial inflow and is not significantly affected by venous compliance (104).

Ultrasound measurements

Baseline red blood cell velocity and diameter of the superficial femoral artery are measured using an Doppler ultrasound device (HDI 5000 Ultrasound System, ATL Ultrasound, USA; Megas, ESAOTE, Italy; ARTLAB system, Pie Medical, The Netherlands; WAKI, Atys Medical, France;) with a 5-7.5 Mhz broadband linear array transducer (105,106). Mean red blood cell velocity (V_{mean}) and systolic and diastolic diameter of the superficial femoral artery are measured, approximately 2 cm distal of the bifurcation, are measured. V_{mean} is calculated as the average of 20 Doppler waveforms. Automated software was used for operator-independent analyses of waveforms. For diameter measurements, the average of 6 consecutive mean diameters is obtained. Leg blood flow is calculated with the following formula: $(\pi \cdot r^2 \cdot V_{mean}) \cdot 60$ (r = ½ diameter of the superficial femoral artery.

The perfused leg model

The technique of leg blood flow measurements in combination with drug infusions into the femoral artery is developed collectively by the departments of physiology and pharmacology-toxicology (78). A cannula (Angiocath 16-gauge, Becton Dickinson, USA) is introduced into the femoral artery of the leg using a modified Seldinger technique. The intra-arterial cannula is used for drug administration (Type P2000, IVAC Medical System, USA) and for blood pressure measurements (HP monitor type 78353B, Hewlett Packard, Germany). Leg blood flow is measured with bilateral venous occlusion plethysmography, as described earlier.

Apart from the aforementioned hemodynamic measurements, we also examined neurotransmitter and humoral parameters. We assessed plasma levels of noradrenaline, adrenaline, renin and angiotensin II. Furthermore, in *chapter 7* we

assessed plasma volume by indicator dilution method using radio-labelled albumin (^{125}I-HSA) (107).

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Leg intravenous pressure during head-up tilt

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ABSTRACT

Leg vascular resistance is calculated as the arterial - venous pressure gradient divided by blood flow. During orthostatic challenges it is assumed that the hydrostatic pressure contributes equally to leg arterial as well as to leg venous pressure. Because of venous valves, one may question whether, during orthostatic challenges, a continuous hydrostatic column is formed and if leg venous pressure is equal to the hydrostatic pressure. The purpose of this study was, therefore, to measure intravenous pressure in the great saphenous vein of 12 healthy individuals during 30° and 70° head-up tilt and compare this with the calculated hydrostatic pressure. The height difference between the heart and the right medial malleolus level represented the hydrostatic column. The results demonstrate that there were no differences between the measured intravenous pressure and the calculated hydrostatic pressure during 30° (47.2 ± 1.0 and 46.9 ± 1.5 mmHg, respectively) and 70° head-up tilt (83.9 ± 0.9 and 85.1 ± 1.2 mmHg, respectively). Steady state levels of intravenous pressure were reached after 95 ± 12 s during 30° and 161 ± 15 s during 70° head-up tilt. In conclusion, the measured leg venous pressure is similar to the calculated hydrostatic pressure during orthostatic challenges. Therefore, the assumption that hydrostatic pressure contributes equally to leg arterial as well as to leg venous pressure during orthostatic challenges can be made.

INTRODUCTION

The increase in leg vascular resistance, by a baroreflex induced increase in sympathetic tone and by local mechanisms such as the veno-arteriolar axon 'reflex' (VAR) (1) and the myogenic response (2), are important to withstand orthostatic challenges (3). Peripheral vascular responses to orthostatic challenges, quantified by leg vascular resistance, have been studied in healthy individuals as well as in individuals with autonomic dysfunction (2,4-14).

In supine position, leg vascular resistance is calculated as the arterial - venous pressure gradient (P_a - P_v) divided by blood flow. In supine position, most studies (2,5-8,10) assume that venous pressure equals 0 mmHg and use mean arterial blood pressure (MAP) to replace the arterial - venous pressure gradient, while others (4,11-14) have estimated venous pressure in the leg using venous occlusion plethysmography. During orthostatic challenges a hydrostatic pressure is added to leg arterial as well as to leg venous pressure due to gravitational translocation of blood (3,15). One may question how upright posture will affect the arterial - venous pressure gradient. The veins have valves that close at the onset of orthostatic challenges and thereby interrupt the development of a continuous hydrostatic column. When an orthostatic challenge persist, the veins will continue to fill with blood and consequently the venous valves will open to form a continuous hydrostatic column (15-17). At this time the assumption can be made that the hydrostatic pressure contributes equally to leg arterial as well as to leg venous pressure (15) and vascular resistance can be calculated as MAP (- P_v supine) divided by blood flow (2,4-14).

Surprisingly, only a few studies have tested this assumption by measuring intravenous pressure. Unfortunately, these studies were done in a small number of individuals (18) and/or during relaxed (motionless) standing (18,19), which still could cause considerable (in)voluntary muscle contractions (19). Recent studies on leg vascular responses use passive head-up tilt (HUT) to diminish these (in)voluntary muscle contractions. Others (4,11,12), have tested the assumption during passive HUT, but they used an indirect, non-invasive method (20) that could only be used in (HUT) angles up to 35°.The purpose of this study was, therefore, to measure leg venous pressure during 30 ° and 70 ° HUT. We hypothesized that the measured leg venous pressure is similar to the calculated hydrostatic pressure and, therefore, that the assumption can be made that the hydrostatic pressure makes an equal

contribution to leg arterial as well as to leg venous pressure during orthostatic challenges.

METHODS

Subjects

Twelve healthy individuals (6 males and 6 females) volunteered to participate in this study (Table 1). Subjects never smoked, were normotensive (< 140/90 mmHg), free of overt cardiovascular diseases and did not report orthostatic intolerance. None of the subjects used any medication, except from oral contraceptives in female subjects. The study has been carried out in accordance with the Declaration of Helsinki (2000) and was approved by the medical ethical committee of our institution. All subjects gave written informed consent.

Table 1. Subject characteristics.

	Mean±SD	Range
Age (yrs)	22 ± 2	20 – 28
Height (cm)	176 ± 6	168 – 187
Weight (kg)	69 ± 7	55 – 82
Calf circumference (cm)	37 ± 1	35 – 39
Systolic BP (mmHg)	119 ± 6	105 – 130
Diastolic BP (mmHg)	72 ± 7	64 – 85
MAP (mmHg)	88 ± 7	78 – 97

Values represent mean±SD. BP, blood pressure. MAP, mean arterial blood pressure.

Experimental procedures and protocol

All subjects refrained from caffeine-containing food and beverages, vitamin C supplements, and alcohol for at least 12 hours prior to the experiment and refrained from heavy physical activity for at least 24 hours prior to the experiment. Room temperature was controlled at 23 ± 1 °C. After completing a health questionnaire, subjects were comfortably placed in the supine position on a manually controlled tilt table with footboard. A chest belt was used to prevent them from falling down in case of vasovagal syncope. The right leg was placed in such a way that the right medial malleolus was on heart level, mid-thoracally at the second intercostal space, in the supine position. The subjects supported their body weight during HUT by standing on the left leg, allowing the right leg to be relaxed for venous pressure measurements.

The experimental procedures started after a supine resting period of at least 30 minutes after placement of an intravenous catheter in the right great saphenous vein.

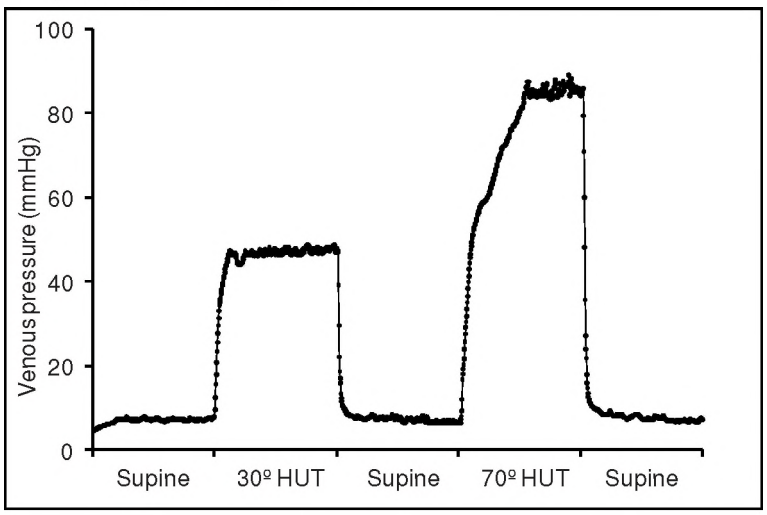
First, baseline venous pressure was measured for 5 minutes in the supine position. Subsequently, subjects were tilted manually, within 5 seconds, to passive 30° HUT position for 5 minutes, during which venous pressure was continuously measured. After returning to the supine position and venous pressure returned to baseline values, the same procedure was repeated with a passive 70° HUT.

Measurements

Blood pressure (BP) was measured auscultatory at the right brachial artery using a sphygmomanometer. Mean arterial blood pressure (MAP) was calculated as diastolic BP + 1/3(systolic BP - diastolic BP).

A 20-gauge venous catheter was placed in the right great saphenous vein at the level of the medial malleolus and connected to a continuous pressure monitoring system (Edwards Life sciences Services Gmbh, Germany). Throughout the entire protocol the venous catheter was fixed in the same position, e.g. in the saphenous vein at the level of the right medial malleolus. Figure 1 shows a typical intravenous pressure recording of one subject. Intravenous pressure (mmHg) was determined as the average of the last minute in each position. The time to reach a steady state intravenous pressure was determined.

Figure 1. A typical intravenous pressure recording of one single subject during the complete experimental protocol.



The hydrostatic column was determined by measuring the vertical height difference (cm) between heart level, midthoracally at the second intercostal space, and the right medial malleolus where intravenous pressure was monitored. To convert to units of mmHg, the height difference was multiplied by a factor 0.766, which includes a correction for the specific gravity of blood (= 1.055) (4,13,21). The determination of the hydrostatic column was done in duplicate, with a coefficient of variation less than 4%.

Statistical analysis

Data are presented as mean ± SEM, unless otherwise stated. A paired Student's t-test was used to determine the effect of HUT and a Pearson correlation coefficient was calculated to determine the correlation between intravenous pressure and calculated hydrostatic pressure. Bland-Altman plots were constructed to show the distribution of the individual data. The level of statistical significance was set at $\alpha = 0.05$.

RESULTS

Supine intravenous pressure

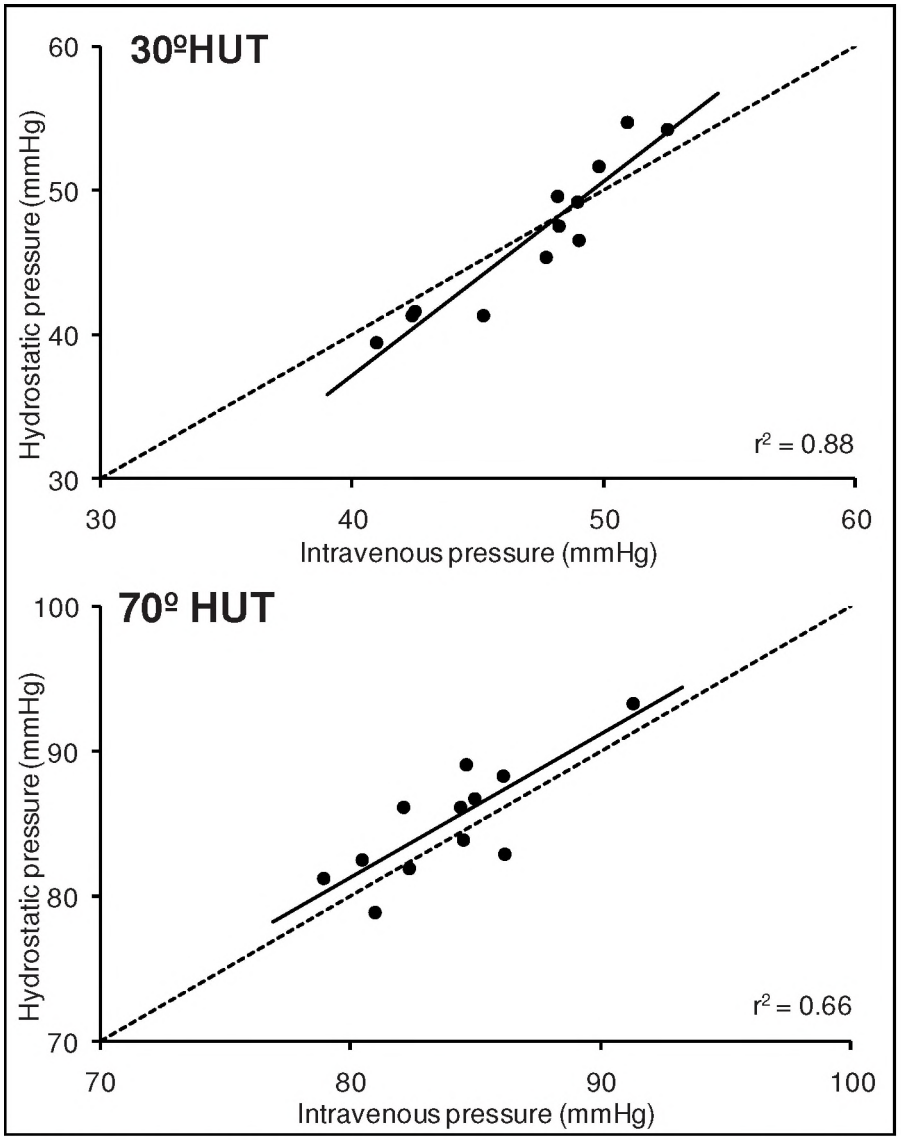
Venous pressure in the great saphenous vein in supine position varied between 6 to 13 mmHg (Table 2), and did not differ between supine positions before 30° and 70° HUT. The mean value of supine venous pressure was 8.7 ± 0.3 mmHg, with a 95% confidence interval (CI) between 8.0 – 9.3 mmHg.

Table 2. Head-up tilt induced changes in intravenous pressure, calculated hydrostatic pressure during head-up tilt and time till steady state intravenous pressure.

	Venous pressure (mmHg)	Hydrostatic pressure (mmHg)	Time till steady state (s)
30° Head-up tilt			
Supine	8.8 ± 0.5		
30° Head-up tilt	47.2 ± 1.0*	46.9 ± 1.5	95 ± 12
70° head-up tilt			
Supine	8.5 ± 0.5		
30° Head-up tilt	83.9 ± 0.9*	85.1 ± 1.2	161 ± 15

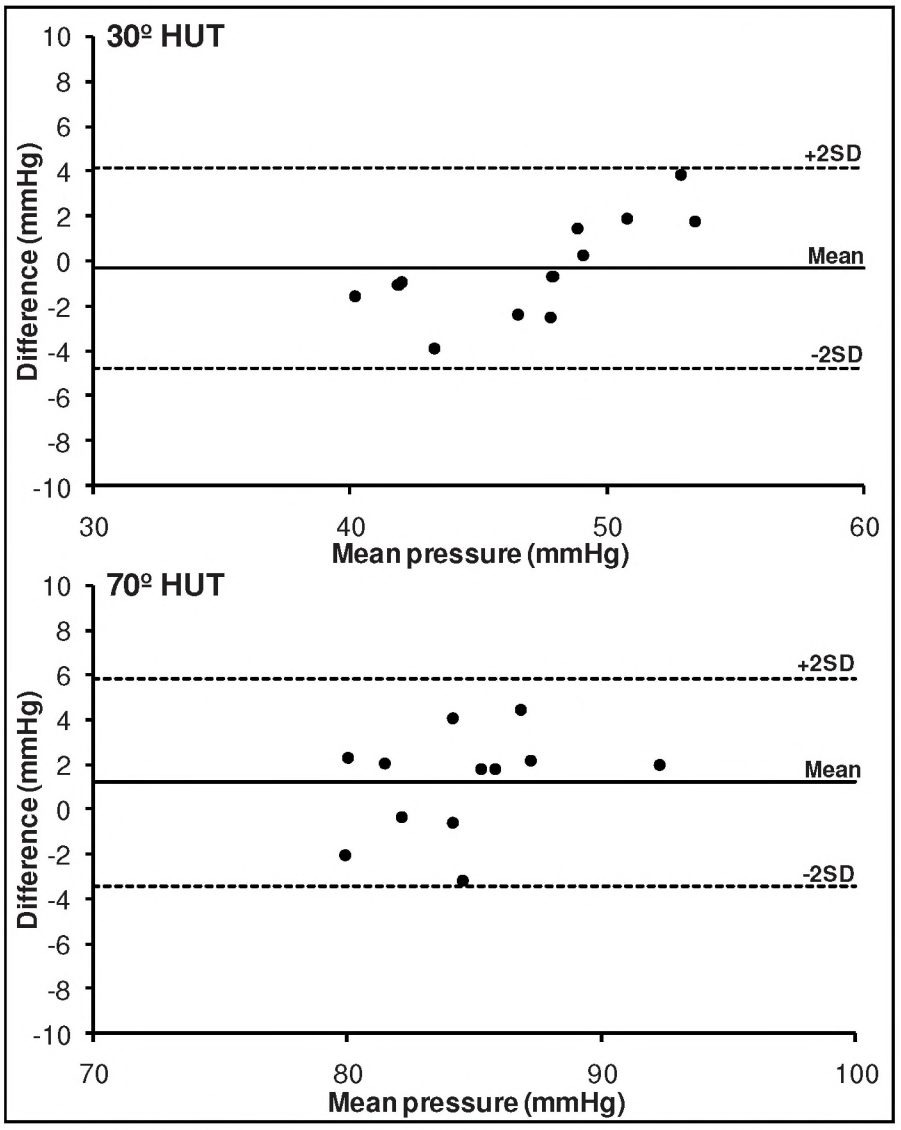
Values represent mean±SE. HUT, head-up tilt. *, significantly different from supine.

Figure 2. Correlation for measured intravenous pressure and calculated hydrostatic pressure for 30° and 70° head-up tilt.



The dashed lines have been constructed to pass through the origin and have a slope of unity (slope = 1.0). HUT, head-up tilt

Figure 3. Bland-Altman analysis of measured venous pressure and calculated hydrostatic pressure for each individual (n=12) during 30° and 70° head-up tilt.



Average of measured venous pressure and calculated hydrostatic pressure (x axis) plotted against difference of calculated hydrostatic pressure minus measured venous pressure (y-axis). The solid horizontal line indicates mean difference and the dashed horizontal lines indicate ± 2SD. HUT, head-up tilt.

Intravenous and hydrostatic pressure during head-up tilt

During both 30° and 70° HUT, the venous pressure increased in the great saphenous vein and reached steady state levels (Table 2 and Figure 1). The intravenous pressures during 30° HUT ranged from 41 to 51 mmHg and during 70° HUT from 80 to 91 mmHg. These steady state levels were reached between 37 and 168 s for 30° and between 64 and 247 s for 70° HUT. The calculated hydrostatic pressures ranged from 39 to 55 mmHg during 30° HUT and from 79 to 93 mmHg during 70° HUT (Table 2). No differences were found between the measured intravenous pressure and the calculated hydrostatic pressure during both 30° HUT (47.2 ± 1.0 and 46.9 ± 4.5 mmHg, respectively) and 70° HUT (83.9 ± 0.9 and 85.1 ± 1.2 mmHg, respectively) (Table 2).

The measured intravenous pressure and the calculated hydrostatic pressure during 30° and 70° HUT correlated well, with a correlation coefficient (r) of 0.937 ($p < 0.001$) for 30° HUT and a correlation coefficient of 0.813 ($p < 0.001$) for 70° HUT (Figure 2). In figure 3 Bland-Altman plots are shown for 30° and 70° HUT, demonstrating the distribution of the individual data.

DISCUSSION

The major finding of the present study is that the measured intravenous pressure in the great saphenous vein corresponds with the calculated hydrostatic pressure during 30° and 70° HUT. So, the assumption that hydrostatic pressure contributes equally to leg arterial as well as to leg venous pressure during orthostatic challenges seems to be correct for both 30° and 70° HUT. Therefore, the arterial - venous pressure gradient during orthostatic challenges is equal to MAP, and leg vascular resistance can be calculated as MAP divided by blood flow.

Supine intravenous pressure

Although most studies (2,5-8,10) assume that supine leg venous pressure equals 0 mmHg, the results of the present study demonstrate that venous pressure ranges between 6-13 mmHg. This must be due to a combination of static and dynamic pressure (16), since the hydrostatic pressure component was alienated through placement of the right leg on heart level. The above mentioned studies (2,5-8,10), therefore, underestimate supine leg venous pressure and as a consequence

overestimate leg vascular resistance, since vascular resistance is calculated as the arterial - venous pressure gradient divided by blood flow. The studies that indirectly derive supine leg venous pressure (4,11-14), do not seem to underestimate leg venous pressure, as previously demonstrated by Christ et al. (21). The measured supine venous pressure in our study is in agreement with a recent study of Calbet et al.(22),who found a leg venous pressure of 7.1 ± 0.1 mmHg (mean ± SD). However, they measured venous pressure in the femoral vein, which may not be compared directly to venous pressures values in the saphenous vein.

Nevertheless, supine leg venous pressure cannot be assumed to equal 0 mmHg because it was 8.7 (8.0 – 9.3) mmHg (mean (95% CI). A venous pressure within this 95% CI should be assumed when leg vascular resistance is calculated in supine position unless leg venous pressure is measured intravenously.

Intravenous pressure during head-up tilt

Intravenously measured leg venous pressure corresponded and correlated well with the calculated hydrostatic pressure in 30° and 70° HUT. It seems, therefore, correct to make the assumption that during an orthostatic challenge the hydrostatic pressure contributes equally to leg arterial as well as to leg venous pressure. Consequently, the leg vascular resistance during orthostatic challenges can be calculated as MAP (- supine P_v) divided by blood flow.

However, two important remarks have to be made to this assumption. First, because of venous valves the forming of a continuous hydrostatic column in the veins is not instantaneously, as compared to the arteries. When an orthostatic challenge persists, the veins will continue to fill with blood and the venous valves will open allowing the formation of a continuous hydrostatic column (15-17). A gradual increase in intravenous pressure was seen directly after the onset of HUT and a steady state, indicating equilibrium, was seen after 37-168 s during 30° and after 64-247 s during 70° HUT. So, the remark has to be made that the assumption can only be used when this equilibrium is achieved, because from that moment on, a continuous hydrostatic column has been formed in the veins.

Secondly, according to the assumption that during orthostatic challenges hydrostatic pressure contributes equally to leg arterial as well as to leg venous pressure, supine leg venous pressure should be added to the calculated hydrostatic pressure. The results of this study, however, demonstrate that leg venous pressure

during orthostatic challenges equals hydrostatic pressure, without adding the supine leg venous pressure. A possible explanation could be that venous tone is reduced during HUT, although this seems unlikely and one would expect the opposite to occur during HUT. Another explanation could be that the decrease in leg blood flow during HUT (5-7,9,10) lowers venous pressure. Although, this is an interesting question the present study was not designed to answer that question.

Consequently, in the calculation of leg vascular resistance during orthostatic challenges, the arterial – venous pressure gradient can be replaced by MAP, without accounting for supine leg venous pressure.

Limitations

One may argue that the pressure in the great saphenous vein, a superficial vein, does not reflect the pressure in the deep veins (21), which drain most of the blood from the lower limbs (23). However, the pressure gradient between the deep and superficial veins is small, because blood is drained through low resistance valves (24). Moreover, venous pressure measured at various cannulation sites varies by 1-2 mmHg, when measured after an equilibration time of 120 seconds (24). We, therefore, believe that the measured venous pressure in the great saphenous vein represents superficial as well as deep venous pressure.

From the Bland-Altman plot for 30° HUT (Figure 3), one could argue that there might be an overestimation of intravenous pressure from the hydrostatic pressure for low pressures only (< 48 mmHg). However, there were no differences between the intravenous and hydrostatic pressure and no systematic errors for both HUT positions. We are, therefore, convinced that the measured intravenous pressure corresponds with the calculated hydrostatic pressure during 30° and 70° HUT.

In this study we did not measure leg arterial pressure, because the hydrostatic column is immediately formed in the arteries (3,15) and the added hydrostatic pressure to leg arterial pressure is calculated in the exact same manner as hydrostatic pressure is calculated in the present study for leg venous pressure (16).

Recommendations

From the results of this study, some important methodological recommendations can be made.

1) We recommend using a supine leg venous pressure of 8.7 (8.0-9.3) mmHg (mean (95% CI) when the measured leg is placed on heart level.
2) The assumption that hydrostatic pressure contributes equally to leg arterial as well as to leg venous pressure during orthostatic challenges is correct. However, supine leg venous pressure should not be added to the hydrostatic pressure and the assumption can only be made after a continuous hydrostatic column is formed, for 30° HUT this is after 3 min and for 70° HUT after 4 min.
3) It is essential that arterial and venous measurements are at the same level compared to the heart, otherwise a correction equal to the hydrostatic column difference should be made.

When these recommendations are taken into account, leg vascular resistance during orthostatic challenges can be calculated as MAP divided by (leg) blood flow.

Conclusion

During orthostatic challenges the leg venous pressure, measured intravenously in the great saphenous vein, is similar to the calculated hydrostatic pressure. Therefore, the assumption can be made that the hydrostatic pressure contributes equally to leg arterial as well as to leg venous pressure. Although this assumption can only be made when a continuous hydrostatic column has been formed in the veins, the time until this column has been formed is depending on the performed orthostatic challenge.

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Leg vascular resistance increases during head-up tilt in paraplegics

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ABSTRACT

Despite loss of centrally mediated sympathetic vasoconstriction to the legs, spinal cord-injured individuals cope surprisingly well with an orthostatic challenge. This study assessed changes in leg vascular resistance following head-up tilt in healthy (C) and in paraplegic (P) individuals. After 10 min of supine rest, subjects were tilted 30° head-up. Mean arterial pressure (MAP) and total peripheral resistance (TPR) increased in C (MAP from 76.7±6.6 mmHg to 80.6±8.2 mmHg; TPR from 1.12±0.26 AU to 1.19±0.31 AU) while both remained unchanged in P. Echo Doppler ultrasound determined red blood cell velocity in the femoral artery, which decreased (P from 18.9±6.2 cm/s to 12.5±4.5 cm/s, P=0.001; C from 16.3±6.2 cm/s to 10.8±5.0 cm/s, P=0.001) and leg vascular resistance, which increased (P from 402±137 AU to 643±274 AU, P=0.001; C from 238±68 AU to 400±122 AU, P=0.003) from supine to upright. The present study shows that independent of supraspinal sympathetic control, humans are able to increase leg vascular resistance and maintain blood pressure during head-up tilt.

INTRODUCTION

Spinal cord-injured individuals have part of their sympathetic nervous system isolated from brain stem control. Consequently, they show an attenuated or absent increase in sympathetic activity during orthostatic challenges (1). Nevertheless, individuals with paraplegia maintain blood pressure during orthostatic stress (2,3), suggesting an increase in peripheral vascular resistance. Sympathetic reflexes below the level of the lesion induce vasoconstriction independent of brain stem control (4,5). Other local mechanisms contributing to vasoconstriction are the veno-arteriolar axon reflex and the myogenic response. The veno-arteriolar reflex is triggered by venous distension of small veins and probably runs through a sympathetic axon, that innervates both an arteriole and a small vein (6), and may be responsible for ~50% of the vasoconstriction elicited during head-up tilt (7). However, plasma noradrenaline, which normally increases by 80–100% from supine to head-up tilt, hardly increases in spinal cord-injured individuals (8). A myogenic response is a muscle vasoconstrictor response to increments in transmural pressure. Imadojemu et al. (9) suggest that the myogenic response might be engaged in the head-up tilt induced leg vasoconstriction in healthy individuals.

Despite the fact that Skagen et al. (10) and Andersen et al. (11) demonstrated a vasoconstrictor response in tetraplegic individuals to head-up tilt, tetraplegics still show a marked drop in mean arterial pressure (MAP) during tilt, the latter in contrast to paraplegic individuals and controls. This study measured changes in the central and peripheral vascular responses following head-up tilt in paraplegic and healthy-control individuals in order to assess the necessity of intact supraspinal sympathetic control to increase in leg vascular resistance.

METHODS

Subjects

Ten healthy male control (C) and eleven paraplegic (P) individuals participated in this study. P (T4-L1 lesions), eight males and three females, had complete spinal cord lesions (AIS A (12)), except for one male who had a sensory incomplete spinal cord lesion (AIS B) and one who had a motor incomplete but non functional spinal cord lesion (AIS C). In all P individuals, sweating was disturbed under the level of the

lesion, indicating loss of autonomic control of the skin. After completion of the study, six P-males and two P-females returned to the laboratory to assess the completeness of the sympathetic lesion by a cold pressor test of the hand and a separate control group was assembled with similar age and gender profile (39±2 years (mean±SD); seven males and two females).

Three P smoked and two used atenolol for hypertension and one used tofranil to control bladder spasms. One C smoked but none used medication, and all exercised between 0 h/week and 5 h/week (Table 1). The study was approved by the Faculty of Ethics Committee and conformed with the principles outlined in the Declaration of Helsinki. All the subjects gave their written informed consent prior to the study.

Table 1. Physical characteristics.

	C (n=10)	P (n=11)
Age (yrs)	31.1±11.5	36.1±9.4
Height (cm)	179±7	182±8
Weight (kg)	72.7±7.2	71.9±15.2
Calf circumference (cm)	37.2±2.8	31.3±3.9*
Spinal Cord Injury duration (yrs)		9.1±6.9

Values represent means±SD. C, control group. P, paraplegic group. *, significantly different from C.

Protocol

All individuals refrained from caffeine, nicotine and alcohol for 3 h prior to the study. They were studied in a quiet room at 24–26° C on a manually driven tilt table with non-skid in order to prevent sliding and P individuals were supported by a strap around their chest. After at least 15 min in the supine position, the subjects were instrumented. Ten minutes in the supine position was followed, in 5 s, by 10 min in a passive 30° head-up tilt position in order to induce a significant cardiovascular response (13). In the common femoral artery, red blood cell velocity and its diameter were measured after 5 min in the supine and upright position by echo Doppler (HDI 5000 Ultrasound System, ATL Ultrasound, Bothell, USA). Heart rate (HR) and MAP were measured by Portapres (TNO, Amsterdam, The Netherlands) and calf volume changes by strain-gauge plethysmography (Instrumental service, UMC, Nijmegen, The Netherlands).

Measurements

Blood flow in the left common femoral artery was measured using a 5 MHz broadband linear array transducer. The transducer was placed 2.0 cm proximal to the bifurcation of the common femoral artery, with a sample size of 1.5 mm. The transducer was kept at an angle below 60° and in the middle of the vessel. Maximal (Vmax) and minimal red blood cell velocity (Vmin) were determined. The systolic and diastolic diameter of the artery were measured and the mean diameter was 1/3 systolic + 2/3 diastolic diameter. The mean red blood cell velocity (Vmean) was ((1/4Vmax) +(1/6Vmin))1/HR) (HR, beats per second) (14). Blood flow in the artery was $1/2(p \cdot r^2 \cdot Vmean)$ (r = 1/2 mean diameter).

The MAP and HR were measured with a finger cuff attached to the middle phalanx of the third finger of the right hand during head-up tilt. Finger arterial blood pressure reflects accurately the intra-arterial pressure changes (15,16). Data were collected beat to beat at 200 Hz and MAP was calculated. Modelflow, a pulse-contour method, was used to calculate stroke volume (SV) (17-19) and cardiac output was SV times HR. Leg vascular resistance was MAP divided by femoral blood flow and total peripheral resistance was MAP divided by cardiac output. Strain-gauge plethysmography used two mercury-filled tubes of silicon rubber around the thickest part of the left calf, while the foot and knee were supported by pillows, and was calibrated electrically (20).

Data analysis

Variables were analysed off-line every tenth second. The average of each variable was calculated from 7.5 min to 9.5 min while supine and upright.

Cold pressor test

The hand was immersed into water of 4° C for 3 min while calf blood flow was measured using venous occlusion plethysmography and MAP by Finapres.

Statistical analysis

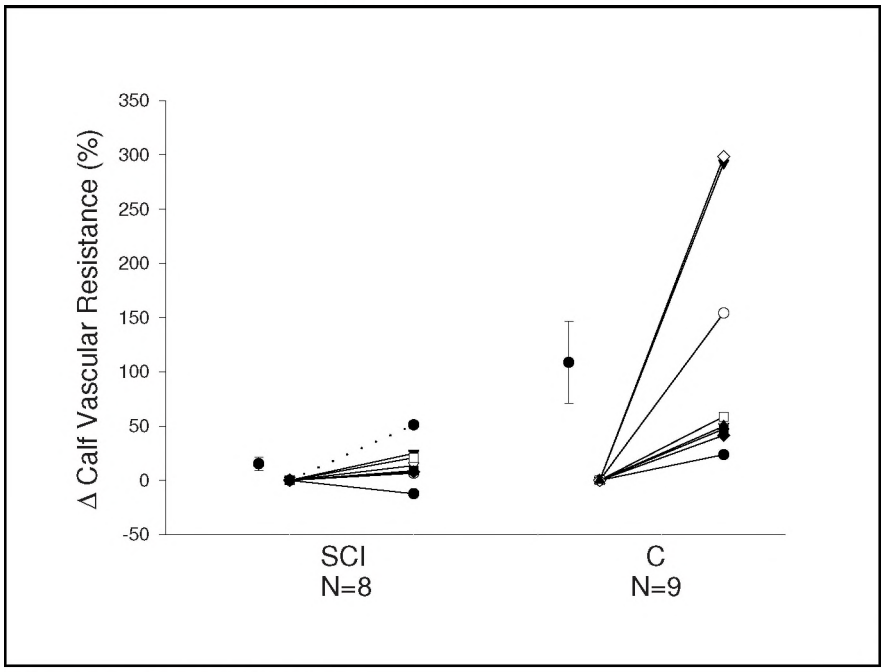
A two-tailed paired Student's t-test was used to assess differences within the groups in vessel diameter, Vmean, blood flow, leg vascular resistance and HR, MAP, SV and total peripheral resistance between supine and head-up tilt. Between the groups the differences in cardiovascular responses from supine to head-up tilt levels were

analysed using ANOVA. P-values less than 0.05 were considered to represent a statistical significant difference.

RESULTS

Individual responses in calf vascular resistance of 8 P and 9 controls are shown in Figure 1. The increase in calf vascular resistance was less in P than in the controls. Three P individuals could not participate in the cold pressor test, but they had complete spinal lesions at midthoracic level. No differences in SV, MAP and total peripheral resistance in supine position were seen between groups, whereas HR was higher in P compared with C (Table 2).

Figure 1. Individual and mean responses of relative calf vascular resistance to the cold pressor test of the hand in spinal cord-injured and control individuals.



SCI, spinal cord-injured. C, controls.

In the supine position, no differences in Vmean were seen between the groups. The diameter of the common femoral artery in P was lower than in C and also leg blood flow was lower and leg vascular resistance higher (Table 3). No differences in diameter

were observed between supine and upright. Since the quality of the measurements was better in the supine position, these diameters were used for all further analysis, both supine and upright.

From supine to head-up tilt, SV decreased 9% in C, but not in P, while HR increased by 9% in C and 5% in P. The MAP in C increased 5%in head-up tilt compared to supine, whereas in P it did not change (Figure 2a). Total peripheral resistance increased 7% in C in head-up tilt compared to supine with no changes in P (Table 2).

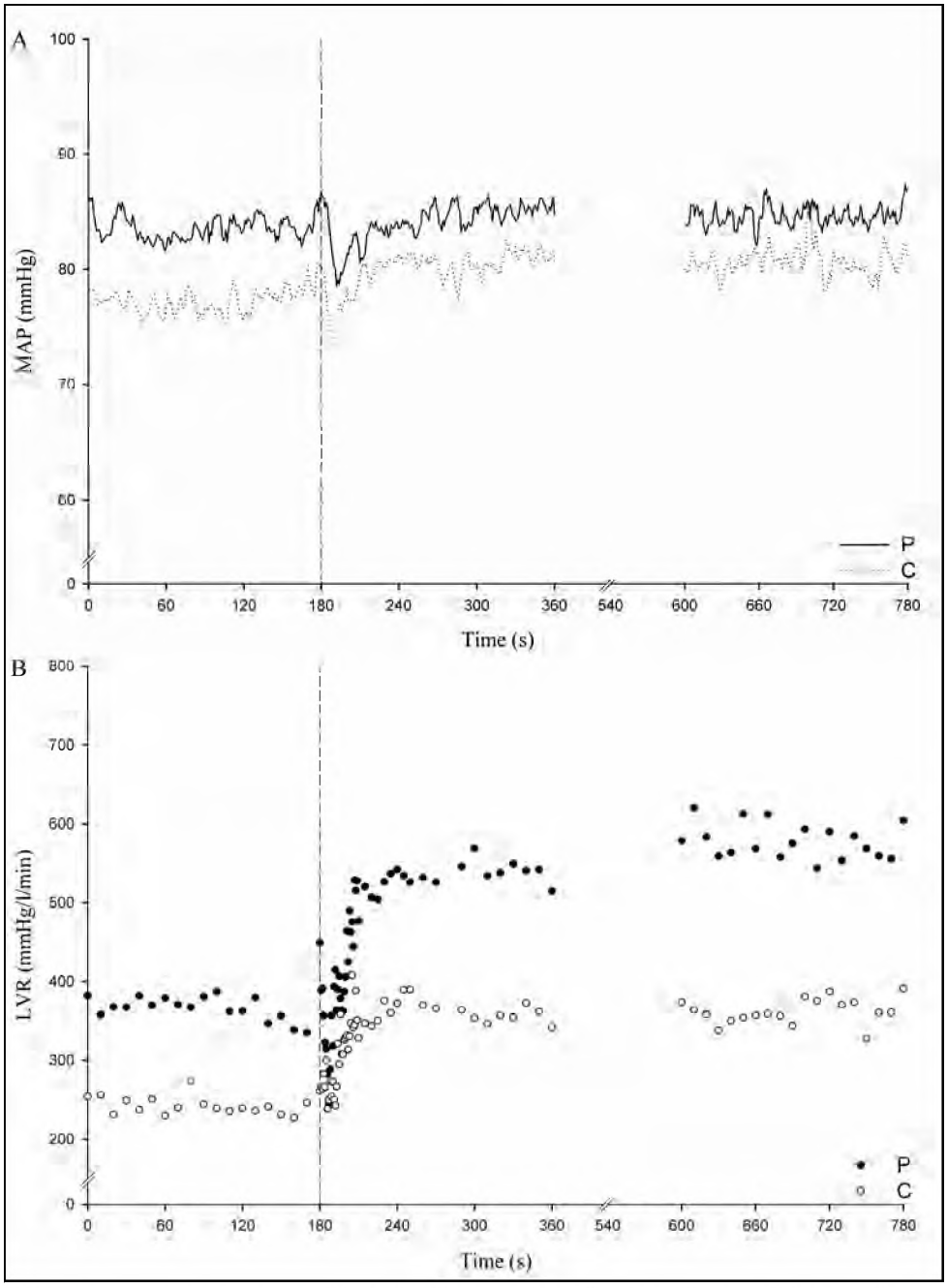
From supine to head-up tilt, leg blood flow decreased in both P (34%) and in C (34%) and leg vascular resistance increased in P (60%) and in C (75%) (Figure 2b). Also, calf volume increased from supine to head-up tilt in P and C (Table 3 and Figure 3).

Table 2. Steady state values of systemic parameters.

		C (n=10)	P (n=11)
Heart rate (bpm)	Supine	58.96.9	68.1±10.7*
	Head-up tilt	64.5±12.0	71.5±8.7
	P-value	0.040	0.036
SV (ml)	Supine	73.3±11.9	70.7±18.0
	Head-up tilt	66.9±10.6	63.0±20.6
	P-value	0.004	0.067
MAP (mmHg)	Supine	76.7±6.6	83.4±12.8
	Head-up tilt	80.6±8.2	84.6±13.2
	P-value	0.027	0.454
TPR (AU)	Supine	1.12±0.26	1.17±0.36
	Head-up tilt	1.19±0.31	1.27±0.47
	P-value	0.028	0.208

Values represent mean±SD. P -values indicate differences between supine and head-up tilt within each group. C, control group. P, paraplegic group. SV, stroke volume. MAP, mean arterial blood pressure. TPR, total peripheral resistance. AU, arbitrary units. *, significantly different from controls.

Figure 2. Mean arterial blood pressure (A) and leg vascular resistance (B) during head-up tilt in paraplegic and controls.



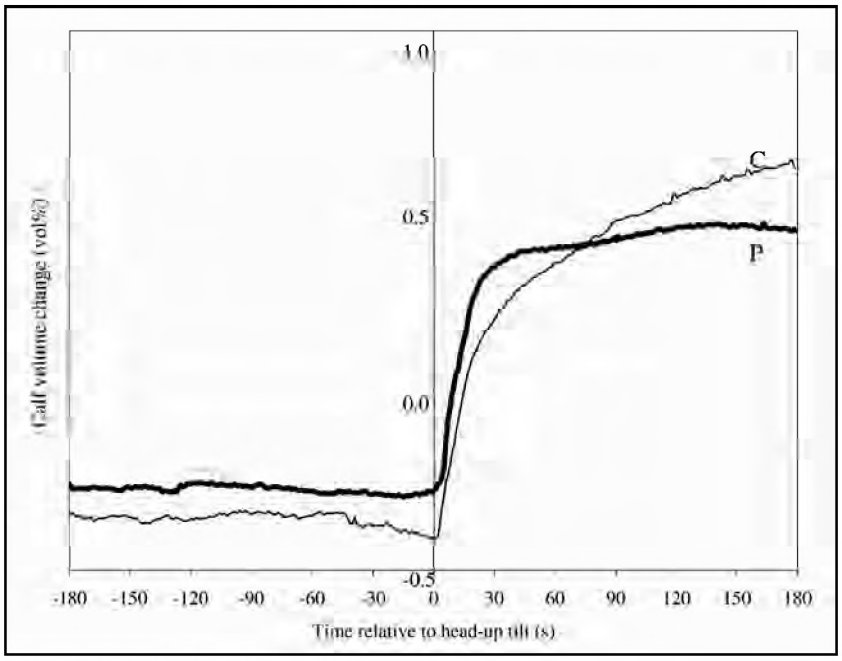
Values represent group-average. The dashed lines represent head-up tilt, preceded by supine rest. MAP, mean arterial blood pressure. LVR, leg vascular resistance. P, paraplegic. C, control.

Table 3. Steady state values of leg variables.

		C (n=10[a])	P (n=11)
Diameter (cm)	Supine	1.01±0.11	0.72±0.13*
Vmean (cm/s)	Supine	16.3±6.2	18.9±6.2
	Head-up tilt	10.8±5.0	12.5±4.5
	P-value	0.001	0.001
Blood flow (l/min)	Supine	0.35±0.09	0.22±0.05*
	Head-up tilt	0.23±0.07	0.15±0.05*
	P-value	0.002	0.001
LVR (mmHg/l/min)	Supine	283.3±68.6	401.6±136.5*
	Head-up tilt	399.5±122.0	642.9±274.1*
	P-value	0.003	0.001
Calf volume change (vol%)	Head-up tilt	1.31±0.70	0.85±0.85
	P-value	0.003	0.040

Values represent mean±SD. P-values indicate differences between supine and head-up tilt within each group. C, control group. P, paraplegic group. V_{mean}, mean red blood cell velocity. LVR, leg vascular resistance. *, significantly different from controls. [a], n=9 for diameter and blood flow in C.

Figure 3. Changes in calf volume upon head- up tilt measured by plethysmography.



P, paraplegic individuals. C, controls.

DISCUSSION

The major finding of the present study is that paraplegic individuals show an increase in leg vascular resistance during head-up tilt comparable with that demonstrated by control subjects.

In healthy individuals, immediately upon head-up tilt, central volume or pressure receptors detect an unloading due to a blood shift from central to peripheral vascular beds (21). The latter is confirmed in the present study by the initial rapid calf volume increase, which results in a reduced venous return and, consequently, in a decrease in SV and, transiently, in MAP. Within a heartbeat, HR increases and within 10 to 12 s, peripheral vasoconstriction is manifest. The response in vascular resistance seems to be of importance to restore MAP, or in C to enhance MAP.

The P individuals were able to increase their leg vascular resistance in the upright posture, despite lack of centrally mediated sympathetic control, and thus, no sympathetic efferent of the baroreflex to the leg blood vessels. The spinal cord injury may have been incomplete or supraspinal control of sympathetic outflow to the leg may have been restored. However, in all P individuals, sweating was disturbed under the level of the lesion, indicating loss of autonomic control of the skin (22). In addition, in 8 of the 11 P individuals, a cold pressor test was performed (23). All these P subjects showed no vasoconstriction in the calf during a cold pressor test of the hand, indicating disrupted supraspinal sympathetic outflow to the leg vasculature.

Blood pressure was maintained at the pre-tilt levels in the P population and this response was not different from C, which confirms data presented by Grimm et al. (2). Leg vasoconstriction in P may result from spinal sympathetic reflex activity, veno-arteriolar reflexes or myogenic responses. As in C, P showed a rapid increase of calf volume (assessed by strain-gauge plethysmography) immediately upon head-up tilt. The shifted blood volume is located mainly in the venous vascular system leading to stretch on the venous vascular walls (24). An increase in pressure of 25 mmHg in the venous vascular system will result in a 40–50% increase in arterial vascular resistance via the veno-arteriolar reflexes, which runs via a sympathetic axon (6,7,25-27). However, against the hypothesis of veno-arteriolar reflexes or spinal sympathetic reflex activity (the latter normally induced by specific triggers like pain and bladder distension rather than by head-up tilt; (28)) seems to be the fact that noradrenaline

hardly shows an increase in P individuals (8) suggesting that the activity of the sympathetic nervous system has not increased. This is in accordance with the lack of an increase in sympathetic activity, as has been assessed by low frequency power in HR variability, in P during head-up tilt (1,2). Crandall et al. (29) showed that the cutaneous veno-arteriolar reflex is not mediated by adrenergic mechanisms but may be due to other local mechanisms. Myogenic mechanisms, associated with changes in vascular pressure, may account to a large extend for the observed vasoconstriction in P. According to Bayliss (30) and Folkow (31), the myogenic response is a potent and powerful vasoconstriction mechanism. With an intact baroreflex mechanism, excitatory influences from extrinsic origin may overrule the slow depolarization response, i.e., the myogenic response. However, when the normal regulatory mechanisms fail, the myogenic response may come into play (31). Imadojemu et al. (9) reported that the myogenic response may contribute in parallel with sympathetic nerve activity to vasoconstriction in healthy individuals during head-up tilt.

Leg vascular resistance rose in both groups, but total vascular resistance only in C, which indicates that the vasoconstriction response in P is a local mechanism triggered by vascular pressure changes, and therefore, not as generalized as in C with an overall vasoconstrictor response. Skagen and Bonde-Petersen (26) showed that proximal nervous blockade in healthy human subjects can block the vasoconstriction in the arms but not in the legs, indicating that leg vasoconstriction is not induced by centrally mediated sympathetic activity.

Besides the neurogenic and myogenic mechanisms, humoral mediated control may contribute to cardiovascular responses upon head-up tilt (32). Ten minutes after 30° head-up tilt, Sander-Jensen et al. (33) found no change in renin, angiotensin, vasopressin, aldosterone and adrenaline in healthy individuals. Wall et al. (34) found no change in vasopressin levels and renin activity during 30° head-up tilt in normal individuals. In tetraplegic individuals, the renin angiotensin system activity is elevated and Mathias et al. (8) showed that renin levels rose more quickly and to higher levels in tetraplegic individuals upon head-up tilt than in healthy controls. Sved et al. (35) as Ozcan et al. (36) showed that vasopressin levels increased rapidly in tetraplegic individuals upon head-up tilt and were doubled within 3 min after tilt. Aldosterone and adrenaline do not appear to play an important role in cardiovascular responses upon head-up tilt in tetraplegic individuals (8). In contrast in P, Wall et al. (34) found

no change in renin and vasopressin activity during 30° head-up tilt. Therefore, a role for humoral-induced vasoconstriction upon tilt in P is less likely.

We speculate that the venous vascular atrophy, as reported in P (37,38), is beneficial for their orthostatic tolerance, although the relationship between venous blood pooling and orthostatic tolerance is controversial (39). Individuals with low thoracic lesions may be able to induce splanchnic vasoconstriction, which will contribute to their orthostatic tolerance, although Halliwil et al. (40) demonstrated that the splanchnic area may not be important in blood redistribution during head-up tilt. However, neither vascular atrophy nor splanchnic innervation, can explain the leg vasoconstriction observed during head up tilt in P.

Vasoconstrictor reserve affects orthostatic tolerance (41,42). Leg vascular resistance in P is higher than in C in the supine position, which implicates that the vasoconstrictor reserve is attenuated in P.

Although we cannot distinguish between veno-arteriolar reflex activity and myogenic responses or other local mechanisms, the results suggest that local regulatory mechanisms come into play and are able to induce leg vasoconstriction when centrally mediated vasoconstrictor mechanisms fail.

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Angiotensin II contributes to the increased baseline leg vascular resistance in spinal cord-injured individuals

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ABSTRACT

Objective: Spinal cord-injured (SCI) individuals demonstrate an increased baseline leg vascular resistance. In addition, despite the lack of sympathetic control, an increase in leg vascular resistance is observed during orthostatic challenges. Based on the vasoconstrictive characteristics of angiotensin II, we examined the hypothesis that angiotensin II contributes to the leg vascular resistance at baseline and during head-up tilt in SCI individuals.

Methods: Supine baseline leg and forearm blood flow were measured using venous occlusion plethysmography and leg blood flow during 30° head-up tilt using duplex ultrasound. Measurements were performed before and 4 hours after an angiotensin II antagonist (irbesartan; 150 mg) in 8 SCI individuals and 8 age- and gender matched able-bodied controls. Vascular resistance was calculated as the arterial-venous pressure gradient divided by blood flow.

Results: Angiotensin II blockade significantly decreased baseline leg vascular resistance in SCI individuals ($p=0.02$) but not in controls, whilst no changes in forearm vascular resistance were found in both groups. Angiotensin II blockade did not alter the increase in leg vascular resistance during head-up tilt in SCI individuals nor in controls.

Conclusion: Our results indicate that angiotensin II contributes to the increased baseline leg vascular resistance in SCI individuals. As angiotensin II does not contribute to forearm vascular resistance, the contribution to leg vascular resistance may relate to the extreme inactivity of the legs in SCI individuals. Angiotensin II does not contribute to the increase in leg vascular resistance during head-up tilt in SCI individuals nor controls.

INTRODUCTION

Angiotensin II plays an important role in the regulation of vascular tone and blood pressure via binding to the angiotensin II subtype 1 (AT_1; vasoconstriction) and 2 (AT_2; vasodilation) receptor (1,2). Whilst angiotensin II does not contribute to vascular tone in healthy controls (3,4), angiotensin II plays a pivotal role in the increased forearm vascular resistance in individuals with an increased renin-angiotensin system (RAS) activity (3-5) (6).

Spinal cord-injured (SCI) individuals demonstrate an increased RAS activity, supported by elevated renin levels (7,8), but also show an increased leg vascular resistance (9-13). Vascular resistance is regulated by various factors, including angiotensin II. Previous studies demonstrated that the increased leg vascular resistance in SCI individuals cannot be explained through the sympathetic nervous system (10) or via endothelium derived nitric oxide (9), whilst endothelin-1 partly contributes to the increased leg vascular resistance (11). In SCI individuals, less angiotensin II is necessary to achieve a similar increase in blood pressure compared with controls (14), which suggests an increased angiotensin II responsiveness in SCI individuals. Angiotensin II might, therefore, play an important role in the increased leg vascular resistance in SCI individuals. SCI individuals offer a unique model of nature to assess peripheral vascular adaptations to inactivity since the skeletal muscles below the lesion are paralyzed and, therefore, extremely inactive. Furthermore, supraspinal sympathetic cardiovascular control is importantly impaired, which could lead to serious complications such as hypotension, orthostatic intolerance and episodic hypertension (autonomic dysreflexia) (15). Understanding the regulation of peripheral vascular tone is of special interest since an increased leg vascular resistance may contribute to the development of pressure sores and pour wound healing due to decreased perfusion in SCI individuals (16).

Despite their spinal cord lesion and concomitant sympathetic disruption, SCI individuals demonstrate an increase in leg vascular resistance during orthostatic challenges to the same extent as controls (12,17). This indicates that in SCI individuals other vasoconstrictive mechanisms contribute to the increase in leg vascular resistance. In SCI individuals, renin levels rise more quickly and to higher levels during orthostatic challenges compared with controls (7,8). Therefore, Angiotensin II

might play a role in the increase in leg vascular resistance during orthostatic challenges in SCI individuals.

The aim of this study was to assess the contribution of angiotensin II to leg and forearm vascular resistance at baseline and during an orthostatic challenge in SCI individuals and controls. We hypothesized that angiotensin II contributes to the increased baseline leg vascular resistance as well as to the increase in leg vascular resistance during an orthostatic challenge in SCI individuals, but not in controls.

METHODS

Subjects

Eight male SCI individuals and eight age- and gender matched able-bodied controls participated in this study (Table 1). All subjects were normotensive (<140/90 mmHg; auscultary blood pressure measurement), free of overt cardiovascular diseases and none of the individuals smoked. Four SCI individuals used medication, none of which is known to substantially interfere with vascular reactivity or the RAS. SCI individuals had longstanding (>5 years) traumatic spinal cord injury with a motor and sensory complete spinal cord lesion (American Spinal Injury Association Impairment Scale (AIS) A (18)) below the fourth thoracic spinal segment (T4). All included SCI individual had a complete paralysis with extremely inactive and deconditioned lower limbs and normal function and activity of the upper limbs. The level of spinal cord injury was assessed by clinical examination. The study was carried out in accordance with the declaration of Helsinki and approved by the medical ethical committee of our institution (Radboud University Nijmegen Medical Centre). All subjects gave written informed consent.

Experimental procedures and protocol

Three days preceding the experiment, subjects followed a sodium-defined diet (2000-2400 mg sodium/day) to control the sodium intake. All subjects refrained from caffeine-containing food and beverages, vitamin C supplements and alcohol for >12 hours and from heavy physical activity for >24 hours prior to the experiment. Subjects fasted for >2 hours and had emptied their bladder in the hour before the experiments. All experiments were performed in the morning in a quiet, temperature-controlled (23±1 °C) room.

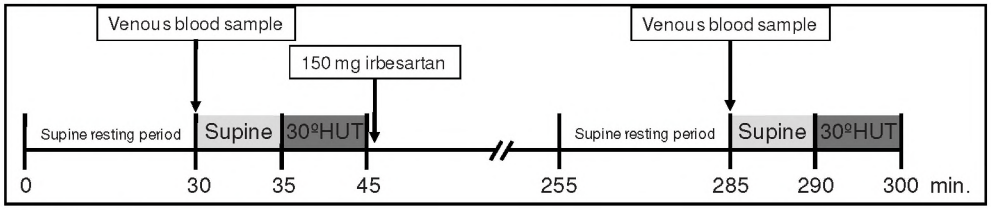
Table 1. Subject characteristics of the spinal cord-injured individuals.

Subject	SCI Level	DOI (yrs)	Age (yrs)	Height (cm)	Weight (kg)	MAP (mmHg)	Medication
SCI 1	T7	17	39	180	84	91	trimethoprim
SCI 2	T11	8	36	185	90	85	
SCI 3	T7	24	55	187	83	104	methenamine, nitrofurantoin, oxazepam
CI 4	T8	14	49	178	78	83	solifenacin
SCI 5	T12	22	45	186	63	95	pregabalin
SCI 6	T6	5	32	173	53	80	
SCI 7	T12	14	46	185	70	106	
SCI 8	T4	6	28	175	75	77	
SCI (n=8)		14±7	41±9	181±5	75±12	90±11	
Controls (n=8)			42±11	179±7	76±9	91±8	

Values represent mean±SD. SCI, spinal cord injury. DOI, duration of injury. MAP, mean arterial blood pressure. T, thoracic spinal segment.

Subjects were positioned comfortably on a manually driven tilt table and supported by a chest belt to prevent them from sliding down during the experiment. The experiment started after a supine resting period of ≥30 minutes. First, baseline leg and forearm blood flow were measured in supine position using venous occlusion plethysmography over a 5-minute period. To examine blood flow responses during orthostatic challenges, we first measured baseline superficial femoral artery blood flow using duplex ultrasound. Subsequently, subjects were tilted manually, within 5 seconds, to a 10-minute passive 30° head-up tilt (HUT). In the last minute of 30°HUT superficial femoral artery blood flow was measured using duplex ultrasound. After the 30°HUT, subjects were returned to the supine position (Figure 1). Subjects then received an oral dose of 150 mg of irbesartan (Aprovel, Sanofi-Aventis), a non-competitive selective angiotensin II subtype 1 (AT_1) receptor antagonist with a high affinity (IC_{50}=1.3 nmol/l) and high mean bioavailability (60-80%) (19,20). The measurements were repeated 4 hours after ingestion of irbesartan, to match the measurements with the peak blood pressure response to irbesartan (21-23). Measurements were performed under the same conditions as described earlier (i.e.>2 hours fasting and bladder emptying >1 hour before the experiments) (Figure 1).

Figure 1. Schematic representation of the experimental protocol.



30°HUT, 30° head-up tilt.

Measurements

Blood pressure was measured continuously using a non-invasive blood pressure device (Nexfin, BMEYE). A finger cuff was attached to the middle phalanx of the right third finger in order to measure finger arterial blood pressure, which accurately reflects intra-arterial blood pressure changes (24). A build-in heart reference system was in operation to correct for hydrostatic influences. Mean arterial blood pressure (MAP) values were derived beat to beat and heart rate was the inverse of the interbeat interval.

Leg and forearm resistance arterial blood flow were measured by ECG-triggered venous occlusion plethysmography, using electrically calibrated (25) mercury-in-silastic strain gauges (Hokanson, Inc.), with a coefficient of variation (CV) between 6-13% (26). In the supine position, the right leg and left arm were positioned ~5 cm above heart level to facilitate venous outflow between venous occlusions (27). Strain gauges were placed around the upper leg, 10 cm above the patella, and around the widest circumference of the forearm. Venous occlusion cuffs, placed on the thigh ~5 cm above the strain gauge and around the upper arm, were simultaneously inflated with a rapid cuff inflator (Hokanson, Inc.), within 1 s, to 50 mmHg (28). Occlusion pressures were sustained for 8 heart cycles after which the cuff was deflated instantaneously (for 10 heart cycles). Blood flow was calculated as the slope of the volume change over a 4-second interval using a customized computer program (Matlab 6.1; Mathworks) (26).

Superficial femoral arterial blood flow during supine rest and 30°HUT was measured using duplex ultrasound, with a CV of 14% (29). Mean red blood cell velocity (Vmean) and systolic and diastolic diameter of the right superficial femoral artery, ~2 cm distal of the bifurcation, were measured with a duplex ultrasound device (ARTLAB system, Pie Medical; WAKI, Atys Medical). Vmean was calculated as the average of 20 Doppler waveforms. Automated software was used for operator-

independent analyses of waveforms (Matlab 6.1; Mathworks). For diameter measurements the average of six consecutive mean diameters was obtained. Real-time automated analyses were performed using the ARTLAB system (Pie Medical). Leg blood flow was calculated with the following formula: $(\pi \cdot r^2 \cdot Vmean) \cdot 60$ ($r = \frac{1}{2} \cdot$ diameter of the superficial femoral artery).

Venous blood samples were drawn from the right antecubital vein in supine rest before and after AT_1 receptor blockade (Fig. 1). Plasma levels of sodium, creatinine, renin and angiotensin II levels were measured before AT_1 receptor blockade and plasma levels of renin and angiotensin II were measured after AT_1 receptor blockade. Plasma renin was measured by immunoradiometric assay (CISbio International) and angiotensin II levels in medium were measured by radioimmunoassay (detection limit 0.5 pmol/l) as described previously (30).

Urine excretion was collected over a 24-hour period preceding the experiment and urine sodium and creatinine concentrations were determined, which enabled the calculation of 24-hour sodium urine output and creatinine clearance.

Data analysis

Leg and forearm vascular resistance were calculated as the arterial-venous pressure gradient (P_a-P_v) divided by blood flow. Supine venous pressure was set at 9 mmHg and during 30°HUT the arterial-venous pressure gradient was replaced by MAP, since hydrostatic pressure makes an identical contribution to leg venous as well as leg arterial pressure (31). Leg and forearm vascular resistance measured using venous occlusion plethysmography are expressed in arbitrary units (AU).

Statistical analysis

Statistical analyses were performed using SPSS 16.0 (SPSS) software. Data are presented as mean±SD, unless otherwise stated. The level of statistical significance was set at $\alpha<0.05$. Independent t-tests were used to assess differences at baseline. Repeated measures ANOVAs were used to examine whether the impact of AT_1 receptor blockade differed between SCI individuals and controls for angiotensin II and renin levels, and leg and forearm vascular resistance. Repeated measures ANOVAs were also used to assess the effect of AT_1 receptor blockade on the increase in leg vascular resistance during 30°HUT within SCI individuals and controls. Post-hoc t-tests were

performed when the ANOVA reported a significant main or interaction effect. Bonferroni's correction was used to correct for multiple comparisons.

RESULTS

Baseline plasma angiotensin II and renin levels were significantly higher in SCI individuals compared with controls (Table 2). AT_1 receptor blockade similarly increased angiotensin II and renin levels in SCI individuals and controls (ANOVA AT_1 blockade p=0.03, interaction p=0.35; AT_1 blockade p=0.01, interaction p=0.40, respectively) (Table 2). Creatinine clearance and 24-hour sodium excretion were similar between SCI individuals and controls, indicating similar sodium load (Table 2).

Table 2. Plasma renin and angiotensin II levels, 24-hour sodium and urine excretion and creatinine clearance in spinal cord-injured and control individuals.

	SCI (n=8)	Control (n=8)	P-value
Renin (mE/l)			
Baseline	19±9	11±8	0.04
AT_1 blockade	103±115*	56±72*	0.17
Angiotensin II (pmol/l)			
Baseline	4.8±1.9	2.7±2.6	0.05
AT_1 blockade	25.9±15.2*	14.9±20.0*	0.13
24-hour sodium excretion (mmol)	164±43	141±43	0.40
24-hour urine excretion (ml)	2545±984	1981±702	0.30
Creatinine clearance (ml/min)	139±45	140±24	0.93

Values represent mean±SD. SCI, spinal cord-injured. AT_1 blockade, angiotensin II subtype 1 receptor blockade with irbesartan. *, post-hoc significantly different from baseline.

Contribution of angiotensin II to baseline vascular resistance

Leg vascular resistance (LVR) was significantly (p=0.01) higher in SCI individuals (39±10 AU) compared with controls (25±8 AU), whereas baseline forearm vascular resistance (FVR) was comparable between both groups (44±13 and 32±17 AU, respectively) (Figure 2). AT_1 receptor blockade significantly (*post hoc* p=0.02) decreased leg vascular resistance in SCI individuals (32±10 AU), but not in controls (29±11 AU) (Figure 2). No changes in forearm vascular resistance were observed after AT_1 receptor blockade in both groups (46±13 and 42±25 AU, respectively) (Figure 2).

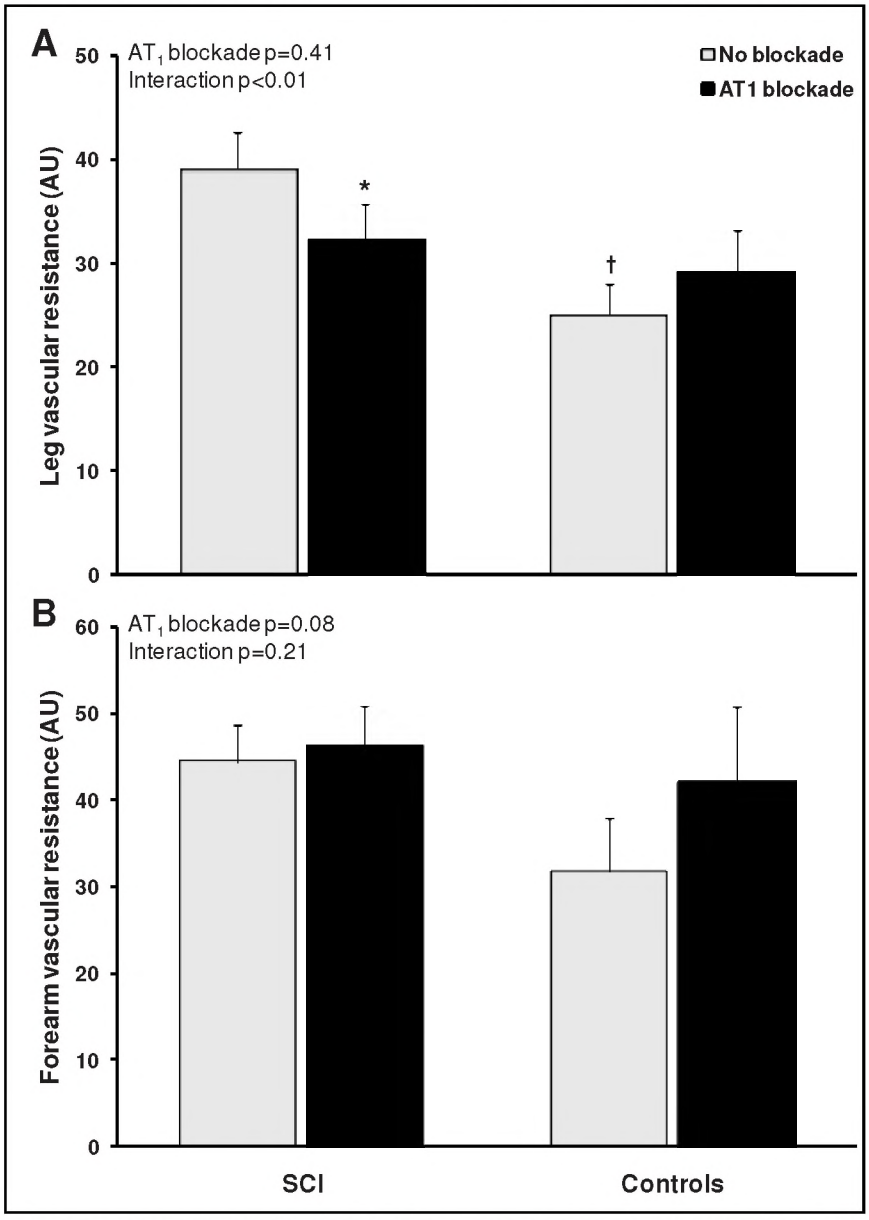
Baseline MAP and heart rate were comparable between groups and did not change after AT_1 receptor blockade (ANOVA AT_1 blockade p=0.07, interaction p=0.29; AT_1 blockade p=0.88; interaction p=0.71, respectively) (Table 3). Leg blood flow was similar between groups and significantly increased (*post hoc* p=0.02) in SCI individuals after AT_1 receptor blockade (ANOVA AT_1 blockade p=0.06, interaction p<0.01) (Table 3). Baseline superficial femoral artery diameter was significantly lower in SCI individuals compared to controls and did not change after AT_1 receptor blockade (ANOVA AT_1 blockade p=0.27, interaction p=0.71) (Table 3).

Table 3. Effect of angiotensin II blockade on leg vascular parameters measured using duplex ultrasound in spinal cord-injured and control individuals in supine and during 30° head-up tilt.

	Position	**SCI individuals (n=8)**		**Controls (n=8)**	
		Baseline	*AT_1 blockade*	*Baseline*	*AT_1 blockade*
SFA diameter (mm)	*supine*	5.3±0.9	5.3±0.9	7.7±0.8†	7.6±0.8†
	30° HUT	5.3±0.7	5.2±0.8	7.5±0.8†	7.5±0.8†
LBF (ml/min)	*supine*	112±64	160±75‡	117±19	106±35
	30° HUT	84±57*	111±58*	80±30*	86±35*
LVR (mmHg/ml/min)	*supine*	1.17±0.83	0.65±0.30‡	0.79±0.14	0.95±0.57
	30° HUT	1.80±1.17*	1.16±0.79*	1.49±0.89*	1.34±0.66*
MAP (mmHg)	*supine*	98±15	96±16	98±8	93±9
	30° HUT	103±22	97±17	100±9	97±9*
Heart rate (bpm)	*supine*	69±10	71±13	59±8	59±10
	30° HUT	73±11*	75±13	62±10	61±9

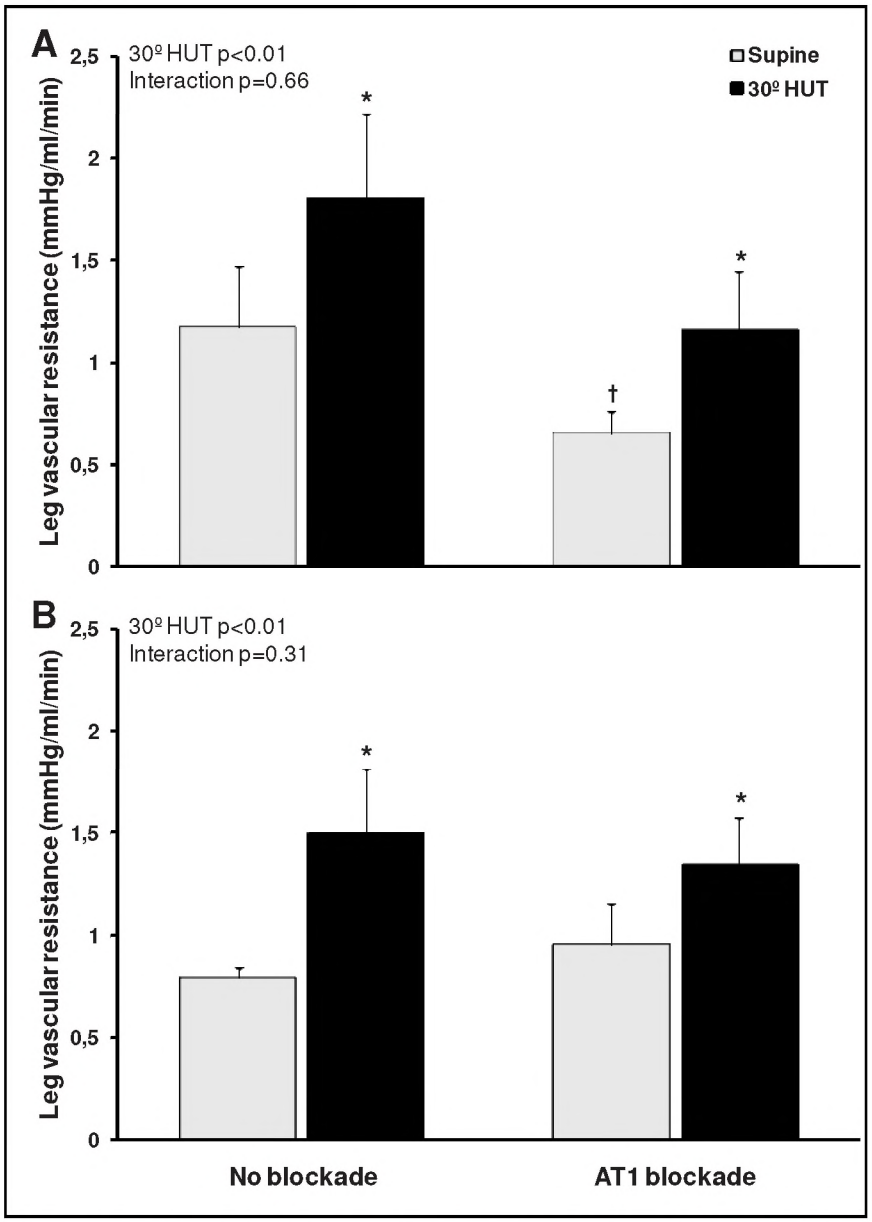
Values represent mean±SD. SCI, spinal cord-injured. AT_1, angiotensin II subtype 1 receptor. SFA, superficial femoral artery. LBF, leg blood flow. LVR, leg vascular resistance. MAP, mean arterial blood pressure. 30° HUT, 30° head-up tilt. *, post-hoc significantly different from supine. †, significantly different from SCI individuals. ‡, post-hoc significantly different from baseline.

Figure 2. Leg (A) and forearm (B) vascular resistance in spinal cord-injured individuals and controls before and after angiotensin II subtype 1 receptor blockade.



Values represent mean+SE. P-values for 2-way repeated measures ANOVA are indicated. SCI, spinal cord-injured. AT_1, angiotensin II subtype 1 receptor. AU, arbitrary units. *, post-hoc significantly different from no blockade. †, post-hoc significantly different from SCI individuals during no blockade.

Figure 3. Supine and 30° head-up tilt leg vascular resistance in spinal cord-injured individuals (A) and controls (B) before and after angiotensin II subtype 1 receptor blockade.



Values represent mean+SE. P-values for 2-way repeated measures. ANOVA are indicated. SCI, spinal cord-injured. AT_1 blockade, angiotensin II subtype 1 receptor blockade. 30° HUT, 30° head-up tilt. *, post-hoc significantly different from supine. †, post-hoc significantly different from no blockade.

Contribution of angiotensin II to changes in vascular resistance during 30° head-up-tilt

30°HUT significantly increased leg vascular resistance in SCI individuals and controls (Figure 3). AT_1 receptor blockade did not alter the increase in leg vascular resistance during 30°HUT in both groups (Figure 3). MAP did not change during 30°HUT in SCI individuals (ANOVA 30°HUT p=0.10, interaction p=0.17), but significantly increased in controls after AT_1 receptor blockade (ANOVA 30°HUT p=0.01, interaction p=0.26) (Table 3). Heart rate significantly increased during 30°HUT in SCI individuals before AT_1 receptor blockade (ANOVA 30°HUT p<0.01, interaction p=0.91), but not in controls (ANOVA 30°HUT p=0.08, interaction p=0.96) (Table 3). Leg blood flow decreased during 30°HUT in both groups and the decrease was comparable before and after AT_1 receptor blockade within SCI individuals and controls (ANOVA 30°HUT p<0.01, interaction p=0.29 and 30°HUT p<0.01, interaction p=0.14, respectively) (Table 3).

DISCUSSION

This study examined the contribution of angiotensin II, a strong vasoconstrictor, to the leg and forearm vascular resistance in SCI individuals and controls. The major findings are that: 1) angiotensin II contributes to the increased vascular resistance of the paralyzed legs, but not to the vascular resistance of the normally innervated forearms in SCI individuals; 2) angiotensin II does not contribute to leg or forearm vascular resistance in controls; and 3) AT_1 receptor blockade does not significantly contribute to the increase in leg vascular resistance during 30°HUT in SCI individuals or controls.

This study is the first to demonstrate a decrease in baseline leg vascular resistance in SCI individuals after selective AT_1 receptor blockade. This indicates that angiotensin II contributes, at least partly, to the increased vascular resistance in the extremely inactive legs of SCI individuals. In contrast, AT_1 receptor blockade did not alter forearm vascular resistance in SCI individuals. These observations demonstrate a localized effect of angiotensin II, which suggests that the extreme physical inactivity in the deconditioned legs of SCI individuals relates to the increased contribution of angiotensin II to vascular resistance. This notion is supported by increased

angiotensin II levels after bed rest (32,33), whereas exercise training reduces angiotensin II levels in heart failure (34) and angiotensin II mediated vasoconstriction in coronary artery disease (35). To further support the idea that physical inactivity relates to the increased vascular resistance, we have consistently demonstrated that electrical stimulation-assisted exercise training of the paralyzed lower limbs in SCI individuals can normalize the vascular adaptations caused by the SCI (11,13,36). Electrical stimulation-assisted exercise could even completely reverses the contribution of endothelin-1 to the increased leg vascular resistance in SCI individuals (11).

Our observations raise questions about the mechanism behind the increased contribution of angiotensin II to the leg vascular resistance in SCI individuals. A potential explanation relates to the elevated angiotensin II and renin levels in SCI individuals compared to controls in our study, a finding similar to previous studies in SCI individuals with high cervical lesions (7,8). However, the circulating angiotensin II levels in SCI individuals were certainly not excessive (37), and angiotensin II blockade did not affect forearm vascular resistance. This argues against a major role for plasma angiotensin II per se. A local phenomenon is therefore more likely to underlie our observations. Angiotensins are known to be produced locally in the vascular wall (38). Such production depends entirely on the uptake of renal renin (37,39). Potentially, the leg vasculature of SCI individuals may display a larger renin uptake compared to the arms. Alternatively, an increase in angiotensin-converting enzyme (ACE) availability could increase the local formation of angiotensin II (39). However, individuals carrying the D-allele of the ACE gene did not have higher angiotensin II levels, despite having 60% higher ACE levels (40). The simplest explanation might be an alteration at the level of the angiotensin II receptors..

Since binding of angiotensin II to the AT_1 receptor mediates vasoconstriction (1,2), an increased sensitivity or density of the AT_1 receptors in the legs would be sufficient to explain our observation in SCI individuals. The exaggerated blood pressor response to angiotensin II in SCI individuals (14) supports this concept. However, a downregulation or a decreased sensitivity of the AT_2 receptors, which mediate vasodilation (2), may also contribute to our findings. Although the effect of inactivity on AT_2 receptors is unknown, exercise training increases AT_2 receptor expression (35). Since vascular changes due to inactivity seem to mirror the effects of exercise, inactivity might decrease AT_2 receptor expression. Future studies should

further examine the exact mechanism explaining the contribution of angiotensin II to the increased leg vascular resistance in SCI individuals.

We recently reported that endothelin-1 contributes to the increased leg vascular resistance in SCI individuals, probably through the endothelin A (ET_A) receptor (11). Interestingly, endothelin-1 and angiotensin II are closely connected. Angiotensin II stimulates the endothelin-1 release in cultured endothelial cells (41) and might mediate hypertensive effects in humans by stimulation of renal endothelium ET_A receptors (42). Furthermore, angiotensin II-induced hypertension in rats can be attenuated by ET_A receptor blockade (43). Whilst angiotensin II and endothelin-1 play an important role in the increased leg vascular resistance in SCI individuals, the link between both vasoconstrictor mechanisms in SCI individuals remains unknown.

Angiotensin II levels did not contribute to baseline vascular resistance in healthy controls. Whilst this finding reinforces previous observations in the forearm of healthy controls (3,4), our data adds to the current knowledge that angiotensin II does not contribute to the leg vascular resistance in healthy controls either. This observation is of special interest, since the magnitude of the effect of endothelium-dependent and independent dilators are limb dependent (44). Suggesting a limb dependent balance between vasoconstrictors and -dilators in controls, which determines the vascular tone in upper and lower limbs.

During orthostatic challenges a baroreflex mediated increase in sympathetic activity will increase heart rate, cardiac contractility and peripheral resistance in order to maintain blood pressure (45). Despite their spinal cord lesion and concomitant sympathetic disruption, our SCI individuals demonstrate a similar increase in leg vascular resistance during 30°HUT compared with controls, which is in agreement with previous studies (12,17). This indicates that other vasoconstrictor mechanisms compensate for the lack of sympathetic control. Although renin levels increase more rapidly in SCI individuals compared with controls (7,8), we found no impact of AT_1 receptor blockade on the vascular responses during 30°HUT in both groups. We studied SCI individuals with (low) thoracic spinal lesions compared to high cervical lesions in previous studies (7,8). All SCI individuals in our study demonstrated increased baseline renin and angiotensin II levels, indicating an increased RAS activity. Our observations indicate that angiotensin II does not contribute to the increase in leg vascular resistance during 30°HUT. One explanation relates to an

insufficient orthostatic stress. However, a 30°HUT causes significant cardiovascular effects (12,17), which are comparable to higher HUT angles (46). Another explanation relates to the duration of our orthostatic challenge (10 minutes), as angiotensin II only contributes to vascular responses during prolonged orthostatic challenges (>25 minutes) in controls (47). During 10-minute orthostatic challenges, angiotensin II does not contribute to the increase in leg vascular resistance in controls (47) and our SCI individuals. The observed increase in leg vascular resistance, therefore, is mediated by other vasoconstrictor mechanisms. The vasoconstrictors endothelin-1, vasopressin and aldosterone unlikely contribute to our observations, since they do not increase or during prolonged orthostatic challenges only (47,48). In a recent study, we demonstrated that the myogenic response, at least partly, contributes to the increase in leg vascular resistance during orthostatic challenges in SCI individuals (17).

Limitations

Since AT_1 receptor blockers for intra-arterial human use are not available, we used an oral selective AT_1 receptor blocker. SCI individuals and controls demonstrated similar increases in angiotensin II and renin levels after ingestion, indicating successful AT_1 receptor blockade. A single dose of irbesartan (150 mg) has a receptor occupancy of ~90% (21,22), which means that AT_1 receptors were not fully blocked. Nonetheless, we found a localized impact on the leg vasculature in SCI individuals.

Our experiments took place in the morning with a 4-hours difference between before and after AT_1 receptor blockade measurements (Fig. 1). The diurnal rhythm of RAS activity, with a peak in the early morning (2-8 a.m.) and a relatively stable plateau during the awake hours(49), could have influenced our measurements. We started the experiments between 9-10 a.m. in both controls and SCI individuals. The diurnal rhythm of RAS activity, therefore, unlikely explains the localized impact of AT_1 receptor blockade on the leg vascular resistance in SCI individuals.

Since venous occlusion plethysmography is suggested to require an empty venous system to guarantee full venous compliance, we measured leg vascular resistance during 30°HUT using duplex ultrasound. In supine position we used venous occlusion plethysmography, since simultaneous measurements of different limbs is possible with a single device. Both methods have a good reproducibility (26,29) and there is a good agreement between the two methods with a correlation coefficient of 0.86 (46). Possible differences at baseline and in the magnitude of

angiotensin II contribution to leg vascular resistance might be explained by the fact that both methods measure a different part of the cardiovascular system. Whilst venous occlusion plethysmography measures blood flow in resistance arteries, duplex ultrasound measures conduit artery blood flow. Distinct adaptations at both levels have been documented in response to exercise as well as physical inactivity (36).

Perspectives

SCI individuals are prone to develop pressure sores and suffer from poor wound healing below the level of the spinal cord lesion, which are associated with the increased leg vascular resistance (16). Based on the vasodilator effect in the paralyzed legs of SCI individuals, AT_1 receptor antagonists may improve or even prevent these secondary complications. Moreover, prolonged treatment may even improve cardiovascular function and health (1). Finally, our results suggest a detrimental role for physical inactivity to increase the angiotensin II-mediated vascular tone. Accordingly, physical inactivity may contribute, at least partly, to the increased angiotensin II-mediated tone in other disease states, such as heart failure and cirrhosis.

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Sympathetic non-adrenergic transmission contributes to autonomic dysreflexia in spinal cord-injured individuals

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ABSTRACT

Autonomic dysreflexia is a hypertensive episode in spinal cord-injured individuals induced by exaggerated sympathetic activity and thought to be α-adrenergic mediated. α-adrenoceptor antagonist have been a rational first choice, nevertheless calcium channel blockers are primarily used in autonomic dysreflexia management. However, α-adrenoceptor blockade may leave a residual vasoconstrictor response to sympathetic non-adrenergic transmission unaffected. The aim was to assess the α-adrenergic contribution and, in addition, the role of supraspinal control to leg vasoconstriction during exaggerated sympathetic activity provoked by autonomic dysreflexia in spinal cord-injured and by a cold pressure test in control individuals. Upper leg blood flow was measured using venous occlusion plethysmography during supine rest and during exaggerated sympathetic activity in 6 spinal cord-injured individuals and 7 able-bodied control individuals, without and with phentolamine (α-adrenoceptor antagonist) and nicardipine (calcium channel blocker) infusion into the right femoral artery. Leg vascular resistance was calculated. In spinal cord-injured individuals, phentolamine significantly reduced the leg vascular resistance increase during autonomic dysreflexia (8±5 versus 24±13 arbitrary units, p=0.04) in contrast to nicardipine (15±10 versus 24±13 arbitrary units, p=0.12). In controls, phentolamine completely abolished the leg vascular resistance increase during a cold pressure test (1±2 versus 18±14 arbitrary units, p=0.02). The noradrenaline increase during phentolamine infusion was larger (p=0.04) in control than in spinal cord-injured individuals. These results indicate that the leg vascular resistance increase during autonomic dysreflexia in spinal cord-injured individuals is not entirely α-adrenergic mediated and is partly explained by non-adrenergic transmission which may, in healthy subjects, be suppressed by supraspinal control.

INTRODUCTION

Autonomic dysreflexia (AD) is a potentially life-threatening episodic hypertension that develops in 80-90% of spinal cord-injured (SCI) individuals with a spinal cord lesion at or above the sixth thoracic spinal segment (T6) (1). AD occurs in these SCI individuals because a large part of the sympathetic nervous system is without central inhibitory pathways (2,3). An arterial pressure increase is induced by exaggerated sympathetic activity caused by visceral, noxious or nociceptive stimuli entering the spinal cord below the level of the lesion and can be initiated by catheterization, bladder distension and bowel evacuation (1-4). AD is accompanied by sweating, flushing and a pounding headache (1,4), and can lead to severe morbidity and even mortality (5-8).

Clinically, AD has been well documented, but the mechanisms that mediate AD remain unclear (9). Because AD is induced by exaggerated sympathetic activity it is thought to be α-adrenergic mediated (2-4). Therapy with an α-adrenoceptor blocker has, therefore, been a rational first choice in AD management (4). However, α-adrenoceptor blockers may leave a residual vasoconstrictor response to sympathetic co-transmitters such as adenosine triphosphate (ATP) and neuropeptide Y (NPY) unaffected (10). These neurotransmitters may still cause a vasoconstrictor response during α-adrenoceptor blockade, although their exact role during exaggerated sympathetic activity is unclear. Because SCI individuals lack supraspinal sympathetic control, their responses could differ from able-bodied individuals in the contribution of non-adrenergic transmission during exaggerated sympathetic reflexes. Nowadays, a calcium channel blocker (nifedipine) is most commonly used as primary agent in the management of AD (4). A calcium channel blocker may be useful to prevent or control AD, indicated by a lower blood pressure response (11). However, the effect of calcium channel blockers on the vasoconstrictor response during AD is unknown.

The first aim was to assess the α-adrenergic contribution to leg vasoconstriction during AD in SCI individuals by α-adrenoceptor blockade. A second aim was to assess whether an α-adrenoceptor antagonist would be more effective than a calcium channel blocker in reducing the leg vasoconstriction during AD. We hypothesized that an α-adrenoceptor antagonist would abolish the leg vasoconstriction during AD and would, therefore, be more effective than a calcium channel blocker. To test this hypothesis the effect of the α-adrenoceptor antagonist phentolamine on leg

vasoconstriction during AD in SCI individuals was compared with the calcium channel blocker nicardipine (intravenous equivalent of nifedipine). The third aim was to assess the role of the presence or absence of supraspinal control on sympathetic non-adrenergic transmission during exaggerated sympathetic activity by comparing the vascular responses of SCI with control individuals. We hypothesized that the contribution of α-adrenergic receptor stimulation would be more pronounced in SCI individuals. To test this hypothesis the effect of phentolamine on leg vasoconstriction during exaggerated sympathetic activity in SCI, by means of AD, was compared with control individuals, by means of a cold pressor test.

METHODS

Subjects

Six male SCI individuals and seven healthy male able-bodied control individuals participated in this study (Table 1). All subjects were normotensive (<140/90 mmHg; auscultary blood pressure measurement), free of overt cardiovascular diseases and did not report orthostatic hypotension. Two SCI and two control individuals smoked, four SCI individuals used medication, none of which are known to substantially interfere with vascular reactivity (rectal laxantia (n=2), furosemide and tolterodine). All SCI individuals had longstanding traumatic spinal cord injury with a motor and sensory complete spinal cord lesion above T6 (AIS A, zone of partial preservation above T6 (12)). The level of spinal cord injury was assessed by clinical examination. The study was carried out in accordance with the declaration of Helsinki and was approved by the medical ethical committee of our institution. All subjects gave written informed consent.

Experimental procedures and protocol

All subjects refrained from caffeine-containing food and beverages, vitamin C supplements, nicotine and alcohol for >12 hours prior to the experiment and from heavy physical activity for >24 hours prior to the experiment. Subjects had been fasting for >12 hours and had emptied their bladder in the hour before the experiment. All experiments were performed in the morning in a quiet, temperature-controlled room (23±1 °C). Each subject was studied on two different occasions, separated by one week. On the first experimental day subjects were screened with a

health questionnaire, physical examination and a resting electrocardiogram. Subsequently, a measurement of leg vascular resistance (LVR) in supine rest and during exaggerated sympathetic activity, i.e. AD in SCI and a cold pressor test of the hand (CPT) in control individuals. On the second experimental day nicardipine, a calcium ion influx inhibitor (calcium channel blocker), and phentolamine, a non-selective competitive antagonist of α-adrenergic receptors, were successively infused into the right femoral artery. LVR was measured during supine rest as well as during AD in SCI and during a CPT in control individuals.

Table 1. Subject characteristics, including the specific characteristics of the spinal cord-injured and control individuals.

Subject	SCI Level	DOI (yrs)	Age (yrs)	Height (cm)	Weight (kg)	SBP (mmHg)	DBP (mmHg)
SCI1	C5	17	46	181	105	130	78
SCI2	T5	28	46	198	65	108	78
SCI3	C7	45	69	179	81	108	60
SCI4	T5	10	39	178	70	106	62
SCI5	C7	34	52	182	92	108	62
SCI6	C7	12	36	183	58	110	50
SCI	n=6		44±2	181±4	78±6	112±3	65±4
C1			22	180	67	118	76
C2			34	194	110	124	90
C3			46	174	65	118	82
C4			26	188	73	128	64
C5			42	188	88	118	78
C6			29	180	76	136	70
C7			54	187	85	138	70
Control	n=7		36±4	184±2	81±5	126±3*	76±3

Values represent mean±SD. SCI, spinal cord-injured individuals. C, control individuals.DOI, duration of injury. SBP, systolic blood pressure. DBP, diastolic blood pressure. C, cervical spinal segment. T, thoracic spinal segment. *, significantly different from SCI.

On both days, subjects were positioned comfortably in supine position on a bed with an anti-ulcer mattress. Experimental procedures on the first experimental day started after a supine resting period of at least 30 minutes. First, baseline upper leg blood flow was measured for 10 minutes in supine position and, subsequently, AD was provoked in SCI individuals for 5 minutes. In control individuals, a CPT of the hand was applied for 3 minutes, during which upper leg blood flow was measured. On the

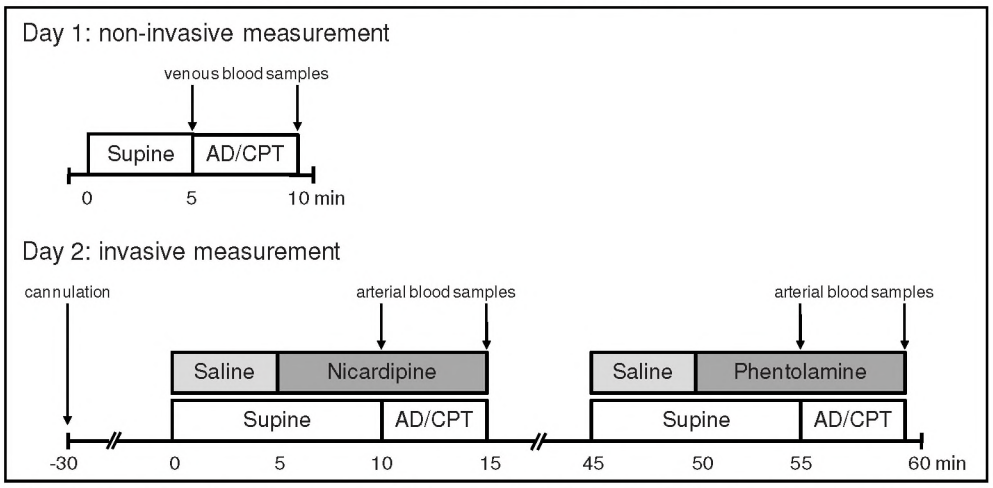
second experimental day an intra-arterial cannula (Angiocath 16 gauge, Becton, Dickinson Infusion Therapy Systems Inc, USA) was introduced after local anesthesia (0.4 ml lidocainehydrochloride 10 mg/ml, Fresenius Kabi Nederland BV, The Netherlands) using a modified Seldinger technique into the right femoral artery at the level of the inguinal ligament for arterial blood pressure measurement (HP monitor type 78353B, Hewlett Packard GmbH, Germany) and intra-arterial drug administration by an automatic syringe infusion pump (Type P2000, IVAC Medical Systems, England). The measurements started after a supine resting period of at least 30 minutes after cannulation of the right femoral artery. First, baseline upper leg blood flow was measured during a 5-minute saline (NaCl 0,9%, Baxter BV, The Netherlands) infusion period, followed by nicardipine infusion (Cardene, 1 mg/ml, Astellas Pharma BV, The Netherlands) during 10 minutes in a dose of 0.5 µg/min/100ml of leg volume (13,14). In a pilot study higher dosages of nicardipine did not further increase blood flow in a control individual. In the last 5 minutes of nicardipine infusion, AD was provoked in SCI and a CPT in control individuals. After a resting period of at least 30 minutes with only saline infusion, the same protocol was performed but this time with phentolamine infusion (Regitine, 10 mg/ml, Novartis Pharma BV, The Netherlands) in a dose of 12 µg/min/100ml of leg volume (15). Nicardipine was infused first because of the short half-life time of 2-5 min (16), in contrast to the unclear half-life time of phentolamine. The schedule of the protocol is shown in figure 1.

AD in SCI individuals was provoked by inflating a blood pressure cuff to 220 mmHg on the contra-lateral upper leg, which gives a nociceptive stimulus (2,3). According to the literature, AD was achieved when there was a systolic blood pressure response to the stimulus of at least 20mmHg (3,17,18) or a 20% increase in blood pressure with visualized vasoconstriction (4). On the first experimental day we attempted to provoke AD with different stimuli (bladder percussion, CPT of the foot and inflating a blood pressure cuff) to see which stimulus would result in the highest increase in blood pressure and would fulfill the criteria (3,4,17,18). Bladder percussion could not be performed in all SCI individuals (sacral rhizotomy and electrical bladder stimulator) and the frequency and pressure of the bladder percussion was difficult to standardize. A CPT of the foot did not increase MAP in 5 consecutive SCI individuals and was therefore considered an inappropriate stimulus. Inflating a blood pressure cuff to 220 mmHg on the contra-lateral leg resulted in the highest increases in blood

pressure, was consistent in provoking AD, appeared easy to standardize and was applicable for each SCI individual. Inflating a blood pressure cuff to 220 mmHg did not result in heamodynamic changes or in LVR of the contra-lateral leg in control individuals. We therefore decided to use this stimulus to provoke AD in SCI individuals.

To elevate sympathetic activity in control individuals, a CPT of the hand was applied (19). A CPT consisted of immersion of the right hand into ice-water (4°C) for a period of 3 minutes (19).

Figure 1. Schematic representation of the experimental protocol.



AD, autonomic dysreflexia in spinal cord-injured (SCI) individuals. CPT, cold pressor test of the hand in control individuals. Saline, NaCl 0,9%. Nicardipine, 0.5 µg/min/100 ml of leg volume. Phentolamine, 12 µg/min/100 ml of leg volume.

Measurements

Bilateral upper leg blood flow was measured by ECG-triggered venous occlusion plethysmography, using mercury-in-silastic strain gauges (D.E. Hokanson, Bellevue, Wash), and electrically calibrated (20). In supine position, the legs were positioned ~5 cm above heart level to facilitate venous outflow between venous occlusions (21). Strain gauges were placed 10 cm above the patella and 12 cm width occlusion cuffs, placed on the thigh above the strain gauge, were inflated with a rapid cuff inflator (D.E. Hokanson), within 1 second, to 50 mmHg (22). Occlusion pressures were sustained for 8 heart cycles after which the cuff was deflated instantaneously (for 10 heart cycles).

Arterial blood pressure was measured continuously using a non-invasive blood pressure device (Portapres, TNO, The Netherlands) on day 1. A finger cuff was attached to the middle phalanx of the left third finger in order to measure finger arterial blood pressure, which accurately reflect intra-arterial blood pressure changes (23). On day 2 arterial blood pressure was continuously measured intra-arterial using the femoral artery cannula. MAP values were derived beat to beat and heart rate was the inverse of the interbeat interval.

Leg volume was determined by anthropometry as described and validated by Jones and Pearson (24).

Venous (day 1) and arterial (day 2) blood samples were taken to determine noradrenaline levels in SCI and control individuals. In SCI individuals, renin and angiotensin II levels were determined as well to establish a possible role of the renin-angiotensin system in AD. The blood samples were taken at rest, before infusion of nicardipine and phentolamine, and directly after AD in SCI and after a CPT in control individuals (Figure 1). The samples were collected in prechilled glass tubes on melting ice containing glutathione and EDTA for determination of noradrenaline levels and non-chilled glass tubes for determination of renin and angiotensin II levels. Samples were processed immediately in a refrigerated centrifuge and stored at -80°C until further analysis. Plasma noradrenaline was measured by sensitive and specific high-performance liquid chromatography (HPLC) with fluorometric detection as described previously (25). Plasma renin was measured by immunoradiometric assay (IRMA) provided by CISbio International (Gif-sur-Yvette, France) and angiotensin II levels in medium were measured by radioimmunoassay (detection limit 0.5 pmol/l) as described previously (26).

Data analysis

A data acquisition system digitalized the data with a sample frequency of 100 Hz (MIDAC, Instrumentation Department, RUNMC, The Netherlands). Upper leg blood flow was calculated as the slope of the volume change over a 4-second interval using a customized computer program (Matlab 6.1; Mathworks, USA). MAP and heart rate values over the same intervals were averaged.

LVR was calculated as the arterial–venous pressure gradient divided by upper leg blood flow. For these calculations, we assumed that central venous pressure was 9

mmHg in supine position (27). During AD and during a CPT the average of the highest three consecutive measurements was taken to determine LVR.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 (SPSS) software. Data are presented as mean±SD, unless otherwise stated. The level of statistical significance was set at $\alpha=0.05$. To assess differences in baseline values between SCI and control individuals unpaired t-tests were used. Repeated measurements ANOVAs were used to assess the effect of AD within the SCI group and of a CPT within the control group on the first day and the effect of infusion of nicardipine or phentolamine on the second day. Post-hoc t-tests were performed when the ANOVA reported a significant main or interaction effect. Bonferroni's correction was used to correct for multiple comparisons.

RESULTS

Baseline values

Supine resting systolic blood pressure and MAP in SCI were lower ($p=0.02$) and LVR was higher ($p<0.01$) compared with control individuals. Provoking AD in SCI and performing a CPT in control individuals on day 1 resulted in an increase in MAP ($p<0.01$ and $p=0.01$, respectively) and LVR ($p<0.01$ and $p=0.01$, respectively), whereas heart rate did not change in either group. The LVR increase during AD in SCI was similar to the increase during a CPT in control individuals (Tables 1, 2 and 3, Figure 2).

Phentolamine and nicardipine infusion during AD in SCI individuals

LVR in SCI individuals was lower ($p=0.02$) during saline infusion on day 2 compared with the baseline value on day 1. Infusion of phentolamine did not change MAP and heart rate, but did lower LVR ($p=0.04$). Provoking AD during phentolamine infusion did not change MAP and heart rate, however LVR increased ($p=0.02$). The increase in LVR was significantly lower ($p=0.04$) during phentolamine infusion (8.0±4.5 AU) compared with the LVR increase on day 1 (23.6±13.4 AU). Nicardipine infusion did not change MAP, heart rate or LVR. Provoking AD during nicardipine infusion resulted in an increase in MAP ($p=0.03$) and LVR ($p=0.04$) with no change in heart

rate. The increase in MAP during AD was significantly lower (p=0.03) during nicardipine infusion (11.9±8.4 mmHg) compared with the MAP increase on day 1 (21.3±10.9 mmHg). Nicardipine did not significantly attenuate the LVR increase during AD (p=0.12), whereas the LVR increase provoked by AD was significantly lower (p<0.05) during phentolamine (8.0±4.5 AU) compared with nicardipine infusion (15.1±9.9 AU). In the non-infused leg no change in LVR was seen during infusion of phentolamine (31.3±10.3 vs. 30.0±10.0 AU), nor during nicardipine (33.0±15.8 vs. 33.8±16.8 AU) (Tables 2 and 3, Figure 2).

Table 2. Systemic hemodynamic variables and leg vascular resistance reactions during autonomic dysreflexia in spinal cord-injured individuals and cold pressure test in control individuals without and with phentolamine or nicardipine infusion.

Intervention	SCI individuals (n=6)			Control individuals (n=7)		
	MAP (mmHg)	*HR (bpm)*	*LVR (AU)*	*MAP (mmHg)*	*HR (bpm)*	*LVR (AU)*
baseline	82±6	55±7	32.7±3.1	96±6‡	57±6	22.3±6.6‡
AD/CPT	103±8*	52±6	56.3±13.8*	112±14*	59±6	40.1±17.8*
Phentolamine infusion						
saline	77±9	51±10	23.8±7.2	90±13	61±7	21.9±5.7
phentolamine	75±12	54±10	18.9±6.2*	86±11	66±8*	12.4±1.9*
AD/CPT	84±11	53±10	26.9±8.2†	91±3	68±4	13.1±2.5
Nicardipine infusion						
saline	*79±10*	*53±11*	*23.6±6.3*	*86±12*	*57±7*	*19.8±5.5*
nicardipine	*78±11*	*53±11*	*21.3±6.0*	*85±11*	*62±8**	*13.8±4.1**
AD/CPT	*90±11†*	*51±10*	*36.4±15.5†*	*94±13†*	*65±4*	*18.1±7.3†*

Values represent mean±SD. SCI, spinal cord-injured. MAP, mean arterial blood pressure. HR, heart rate. LVR, leg vascular resistance. AD, autonomic dysreflexia. CPT, cold pressor test. AU, arbitrary units. *, significantly different from baseline or saline. †, significantly different from nicardipine or phentolamine. ‡, significantly different from SCI individuals.

Phentolamine and nicardipine during CPT in control individuals

Phentolamine infusion in control individuals did not change MAP but did increase heart rate (p=0.02) and resulted in a decrease in LVR (p=0.01). Phentolamine infusion completely abolished the LVR increase in response to a CPT without affecting MAP or heart rate. Infusion of nicardipine did not change MAP but increased heart rate (p=0.02) and decreased LVR (p<0.05) in control individuals. A CPT during nicardipine infusion increased MAP and LVR (p=0.04 and p=0.03, respectively) with

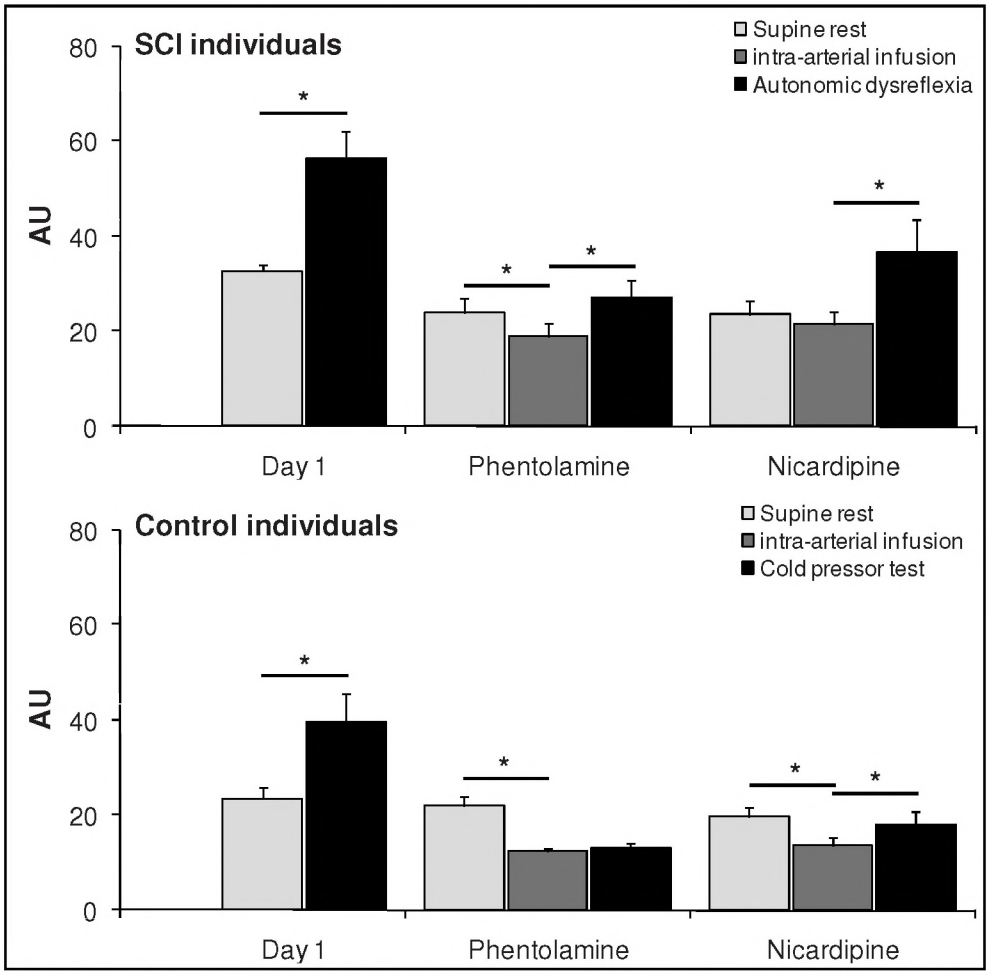
no change in heart rate. The MAP and LVR increase were significantly less pronounced (p=0.04 and p=0.03, respectively) during nicardipine infusion compared with day 1. In the non-infused leg no change in LVR was seen during phentolamine infusion (23.0±8.6 vs. 21.1±8.0 AU) and the LVR increase during a CPT to 27.2± 11.9 AU was lower compared to day 1, probably due to spill-over of phentolamine to the non-infused leg. LVR in the non-infused leg did not change during nicardipine infusion (21.1±7.6 vs. 21.8±8.0 AU) and during a CPT the LVR increased (37.2± 16.0 AU) similar to day 1. (Tables 2 and 3, Figure 2).

Table 3. Individual increase in mean arterial blood pressure and leg vascular resistance during autonomic dysreflexia in spinal cord-injured individuals and cold pressure test in control individuals without and with phentolamine or nicardipine infusion.

Subject	Day 1		Phentolamine infusion		Nicardipine infusion	
	Δ MAP mmHg (%)	*Δ LVR AU (%)*	*Δ MAP mmHg (%)*	*Δ LVR AU (%)*	*Δ MAP mmHg (%)*	*Δ LVR AU (%)*
SCI during AD						
SCI1	12 (13)	4 (13)	2 (2)	14 (87)	8 (8)	9 (57)
SCI2	15 (16)	43 (142)	4 (4)	3 (16)	9 (10)	9 (50)
SCI3	33 (44)	20 (54)	8 (12)	9 (30)	17 (25)	21 (72)
SCI4	6 (8)	19 (67)	1 (2)	10 (64)	4 (5)	24 (101)
SCI5	30 (40)	33 (95)	36 (58)	11 (58)	27 (39)	25 (98)
SCI6	29 (36)	22 (67)	3 (4)	3 (14)	8 (11)	2 (12)
SCI (n=6)	21±11 (26±15)	24±13 (73±40)	9±13* (14±22)	8±5* (45±27)	12±8* (16±13)	15±9† (65±30)
Control during CPT						
C1	4 (5)	7 (51)	8 (10)	1 (9)	13 (16)	11 (58)
C2	14 (15)	8 (54)	-15 (-14)	-3 (-23)	13 (12)	5 (27)
C3	9 (9)	12 (41)	6 (7)	3 (26)	3 (4)	1 (13)
C4	6 (6)	14 (54)	10 (13)	2 (19)	-2 (-3)	1 (8)
C5	15 (15)	17 (58)	6 (7)	0 (3)	6 (7)	1 (10)
C6	34 (36)	47 (175)	14 (19)	0 (3)	16 (21)	10 (82)
C7	31 (32)	21 (110)	7 (9)	0 (4)	13 (16)	1 (14)
Control (n=7)	16±12 (17±12)	18±13 (78±45)	5±9* (7±10)	1±2* (6±14)	9±6* (10±8)	4±4* (30±26)

Values represent mean±SD. SCI, spinal cord-injured individuals. C, controls individual. MAP, mean arterial blood pressure. LVR, leg vascular resistance. Δ, increase. %, percentage increase. AU, arbitrary units. *, significantly different from day 1. †, significantly different from phentolamine infusion.

Figure 2. Leg vascular resistance in spinal cord-injured individuals (n=6, upper graph) and control individuals (n=7, lower graph).



Leg vascular resistance during supine rest, infusion of phentolamine and nicardipine and during autonomic dysreflexia in spinal cord-injured (SCI) and cold pressor test in control individuals with and without infusion. Values represent mean+SE. AU, arbitrary units. *, significant difference.

Blood samples

Baseline noradrenaline levels were significantly lower ($p<0.01$) in SCI compared with control individuals. During AD noradrenaline did not significantly increase in SCI in contrast to control individuals during a CPT ($p=0.01$). Noradrenaline increased significantly during AD in combination with phentolamine and nicardipine infusion in SCI ($p=0.04$ and $p=0.03$, respectively) and during a CPT in control individuals

($p<0.01$, both conditions). The increase in noradrenaline during phentolamine infusion was significantly more pronounced ($p=0.04$) in control than in SCI individuals. There were no significant increases in renin and angiotensin II levels during AD in SCI individuals without or with phentolamine or nicardipine infusion (Table 4).

Table 4. Noradrenaline, renin and angiotensin II levels at baseline and during autonomic dysreflexia in spinal cord-injured and a cold pressure test in control individuals without and with phentolamine or nicardipine infusion.

Intervention	SCI individuals (n=6)			Control (n=7)
	NA (nmol/l)	*Renin (mE/l)*	*AT II (pmol/l)*	*NA (nmol/l)*
baseline	0.66±0.31	10±5	1.96±1.00	1.09±0.18†
AD/CPT	0.93±0.21	10±7	2.02±1.38	1.93±0.71*†
Phentolamine infusion				
saline	0.76±0.74	9±4	1.20±0.32	1.14±0.29
AD/CPT	1.49±1.44*	9±4	1.46±0.58	3.22±1.11*
Nicardipine infusion				
saline	0.60±0.60	7±5	1.34±0.65	0.90±0.15
AD/CPT	1.00±0.92*	7±5	0.96±0.47	1.67±0.41*

Values represent mean±SD. Control, control individuals. NA, noradrenaline. AT II, angiotensin II. AD/CPT, autonomic dysreflexia in SCI individuals or cold pressure test in control individuals. *, significantly different from baseline or saline. †, significantly different from SCI individuals.

DISCUSSION

The major finding of this study is that AD in SCI individuals is not entirely mediated through the α-adrenergic pathway, indicated by presence of a remaining residual leg vasoconstrictor response during AD in SCI individuals during phentolamine infusion. Nevertheless, a more pronounced attenuation of the LVR increase during AD was present during phentolamine infusion compared with a nicardipine infusion. In contrast to SCI individuals, phentolamine infusion completely abolished the vasoconstriction response to a CPT in control individuals which could indicate that the presence of supraspinal sympathetic control in control individuals may suppress the role of non-adrenergic transmission during exaggerated sympathetic activity.

Contribution of non-adrenergic transmission

The LVR increase during AD in SCI individuals did not differ from the LVR increase during a CPT in control individuals. Nevertheless, phentolamine completely abolished the LVR increase in control individuals during a CPT, whereas a significant residual leg vasoconstrictor response occurred in SCI individuals during AD with phentolamine infusion. These results suggest that non-adrenergic transmission contributes to the leg vasoconstriction during exaggerated sympathetic activity in SCI but not in control individuals. This interpretation is supported by the more pronounced increase in plasma noradrenaline levels in control individuals compared with SCI individuals in response to exaggerated sympathetic activity. This observation challenges the widespread view that the vasoconstrictor response during AD in SCI individuals is entirely α-adrenergic mediated (2-4,28).

Since phentolamine is a non-selective competitive α-adrenoceptor antagonist, incomplete α-adrenoceptor blockade could, in theory, explain the residual LVR increase in SCI individuals. A similar intra-arterial dose of phentolamine has previously been used and achieved a maximal vasodilator effect in both SCI and control individuals indicating complete α-adrenoceptor blockade (15,29,30). Moreover, the LVR increase during a CPT in control individuals was completely abolished during phentolamine infusion, despite a larger increase in plasma noradrenaline compared with SCI individuals. This confirms that intra-arterial infusion of phentolamine achieved effective intrasynaptic drug concentrations (30) and excludes incomplete α-adrenergic blockade as an explanation for the residual vasoconstrictor response in SCI individuals in the present study.

Since vasoconstriction during AD occurs rapidly, it is not likely that other vasoconstrictor mechanisms play an important role, such as the renin-angiotensin system (RAS). The RAS is a slow acting vasoconstriction mechanism and, therefore, unlikely to cause immediate arterial vasoconstriction upon a visceral, noxious or nociceptive stimulus. Moreover, there was no increase in renin and angiotensin II levels during AD in SCI individuals supporting the notion that there was no activation of the RAS. However, the local angiotensin system could still play a role since we did not have an additional angiotensin II subtype I receptor blockade. It is unlikely that vasopressin plays a role since vasopressin levels are low in SCI individuals and do not increase during AD (31). The instant reaction to the triggering stimulus provoking AD in combination with the lack of supraspinal control in SCI individuals causing

exaggerated sympathetic activity provides enough evidence for a sympathetic mediated mechanism. Since α-adrenoceptor blockade did not abolish the LVR increase during AD, other sympathetic neurotransmitters might be involved, such as ATP and NPY (10). ATP, NPY and noradrenaline are co-stored in the sympathetic synapse and are simultaneously released (32). Receptors for these neurotransmitters are located on smooth muscle and endothelial cells of blood vessels (32). It is thought that noradrenaline and ATP have a coordinated action in neurogenic vasoconstriction which is modulated by NPY (32). We did not investigate the mechanism by which spinal cord injury increases sympathetic non-adrenergic transmission. Since the sympathetic nervous system below the lesion in SCI individuals is without central inhibitory pathways (2,3), in contrast to control individuals, we speculate that an intact supraspinal control of sympathetic outflow preferentially suppresses sympathetic non-adrenergic transmission. Alternatively, spinal cord injury alters the relative concentrations of co-stored neurotransmitters. In this regard, an intermediate role for endothelin is worth mentioning. We have previously shown that the contribution of endothelin in LVR is increased in SCI individuals (33). In rats endothelin-1 infusion increased the relative contribution of ATP as a functional sympathetic neurotransmitter (34). Therefore, endothelin may mediate the increased contribution of non-adrenergic neurogenic vasoconstriction in SCI individuals.

Phentolamine effect superior to nicardipine

Provoking AD in SCI individuals during nicardipine infusion still resulted in a LVR increase with a concurrent increase in MAP. However, the MAP increase with nicardipine infusion was lower compared with day 1, indicating an attenuation of the blood pressure response during AD in SCI individuals. This is consistent with one previous study demonstrating a lower blood pressure response during oral nifedipine pre-treatment in SCI individuals who exhibited AD during electro-ejaculation (11). However, the LVR increase was similar to day 1 indicating a minor effect of nicardipine on leg vasoconstriction.

Despite the residual LVR increase during AD in SCI individuals with phentolamine infusion, this LVR increase was significantly lower than with nicardipine infusion and, moreover, with phentolamine there was no change in MAP. These results indicate a superior effect of an α-adrenoceptor antagonist compared

with a calcium channel blocker on leg vasoconstriction and concurrent blood pressure response during AD in SCI individuals.

An obvious explanation for the observed difference in effect could be an inefficient dosage of nicardipine. Infusion of nicardipine did not result in a significant decrease in basal LVR in SCI individuals. However, in control individuals basal LVR decreased with the same dose and in a pilot study higher dosages of nicardipine did not result in a more pronounced vasodilatory effect. We are, therefore, convinced that we used a sufficient dose of nicardipine. The vasodilatation caused by nicardipine is, however, more pronounced in hypertensive compared with normotensive individuals (16). Since blood pressure in SCI individuals was lower compared with control individuals the effect of nicardipine on LVR could be smaller for this reason.

Baseline values

Consistent with our results, SCI individuals with a high spinal cord lesion are prone to low resting blood pressures (1-3), which is thought to be due to a diminution in sympathetic nervous activity below the lesion as supported by low plasma noradrenaline levels (1,2). Although the reduced noradrenaline levels in SCI individuals may also be influenced by the efficiency of the re-uptake by the noradrenaline transporter and changes in blood flow redistribution caused by inactivity and muscle atrophy. Despite lower sympathetic activity, SCI individuals have a higher resting LVR (15,30,35), probably due to a combination of functional (33) and structural vascular changes (36). During AD, SCI individuals can increase their LVR to the same extent as control individuals during a CPT. Although a CPT is not directly comparable with AD, both are strong sympathetic stimuli, notwithstanding the physiological and neurological differences, especially the absence (SCI) or presence (control) of supraspinal control (2,3).

Limitations

Although α-adrenergic responsiveness deteriorates with age (37,38), all SCI and control individuals demonstrated similar increases in LVR during AD and a CPT, respectively, compared with their peers. Moreover, the oldest control individual (C7, 54 yrs) had a complete abolished LVR increase during a CPT with phentolamine infusion and the oldest SCI individual (SCI3, 69 yrs) had an attenuated LVR increase during AD with phentolamine infusion.

Infusion of saline in SCI individuals lowered supine LVR compared with day 1. This is probably due to a more pronounced reaction to an increase in shear on the vascular wall, caused by the saline infusion, due to functional changes in SCI individuals (33). The non-infused leg did not demonstrate a lower supine LVR, indicating that the lower supine LVR in the infused leg is probably caused by the saline infusion. The lower supine LVR did not have any effect on MAP and heart rate. Since the focus of the study was the effect of phentolamine and nicardipine infusion on the LVR increase during AD and a CPT, we may assume that the lower basal LVR did not influence our results.

Perspectives

The management of AD in SCI individuals remains a challenge in clinical practice (18,39). Over the years, many different anti-hypertensive agents have been used (4,18). In earlier days α-adrenoceptor antagonists were used (4), however, their use was limited and nowadays nifedipine (calcium channel blocker) is used as a primary agent in the management of AD (4). However, the present study demonstrates that an α-adrenoceptor antagonist appears to have a more pronounced effective than a calcium channel blocker on the LVR and concurrent MAP increase during AD in SCI individuals. Moreover, serious adverse reactions after the use of immediate release nifedipine in hypertensive emergencies in non-SCI individuals have been reported (40). The present study demonstrates that AD in SCI individuals is not entirely α-adrenergic mediated and that sympathetic non-adrenergic transmission may partly explain the LVR increase. The development of antagonists of non-adrenergic transmitters may be a target for future AD management in SCI individuals. Perhaps these antagonists may reduce the blood pressure increase during AD without lowering the blood pressure in the absence of AD.

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Attenuated peripheral vasoconstriction during an orthostatic challenge in older men

6

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ABSTRACT

Background Orthostatic hypotension is common in older men and associated with morbidity and mortality. During orthostatic challenges, older men maintain their blood pressure by an augmented increase in total peripheral resistance. Changes in the leg vascular bed contribute importantly to blood pressure regulation during orthostatic challenges, partly because of blood pooling in the legs. Little is known about the contribution of the leg vascular bed to the augmented increase in total peripheral resistance.

Objective To examine tilt-induced peripheral vasoconstriction in the leg vascular bed of young and older men.

Methods We measured forearm and calf blood flow in 12 young and 12 older men, using venous occlusion plethysmography during 30° head-up tilt (HUT). Forearm and calf vascular resistance were calculated as mean arterial blood pressure divided by blood flow.

Results During HUT, calf and forearm vascular resistance increased in older and young men. The increase in forearm vascular resistance was similar between older (40±6 %) and young men (51±12 %). However, the increase in calf vascular resistance was lower in older (96±15 %) than in young men (175±30 %).

Conclusion Advancing age leads to an attenuated tilt-induced increase in calf vascular resistance, which may contribute to age-related orthostatic hypotension.

INTRODUCTION

Orthostatic hypotension is a common feature with advancing age, with a prevalence around 17 percent in persons aged 65 years and older (1,2), and is associated with falls (2), cognitive decline (3), cardiovascular morbidity (4) and mortality (2,5,6).

During orthostatic challenges venous blood is pooled in the lower limbs and abdomen, leading to a decreased venous return and a transient decrease in cardiac output and blood pressure. The drop in blood pressure activates the baroreflex, resulting in an increase in sympathetic activity. As a consequence, heart rate and vascular resistance are increased to restore cardiac output and blood pressure (7). In contrast to young men, during an orthostatic challenge older individuals demonstrate an attenuated cardiac output (8-10) which is compensated by an augmented increase in total peripheral resistance in order to maintain blood pressure (8-11).

Because venous blood pooling plays a major role during orthostatic challenges, changes in vascular resistance in the splanchnicus and lower limb vascular bed contribute importantly to the blood pressure regulation during orthostatic stress (7). Minson et al. (11), demonstrated an augmented increase in splanchnic vascular resistance during head-up tilt (HUT) in older compared with young men, which may account for the age-related augmented increase in total peripheral resistance. In addition, the lower limb vascular bed is also an important contributor to blood pressure regulation during orthostatic challenges (7). Nonetheless, changes in leg vascular resistance during an orthostatic challenge in older men have never been measured.

The purpose of this study is, therefore, to examine the tilt-induced peripheral vasoconstriction in the leg vascular bed of healthy young and healthy older men. We tested the hypotheses that the tilt-induced leg vasoconstriction is augmented in older men compared with young men, and thereby contributing to the previously reported augmented tilt-induced increase in total peripheral resistance.

METHODS

Subjects

Twelve healthy young (20-28 years) and twelve healthy older men (67-80 years) volunteered to participate in this study (Table 1). Subjects never smoked or stopped

smoking at least 2 years ago. All subjects were normotensive (< 140/90 mmHg), had no signs of varicose veins and were free of overt chronic cardiovascular diseases as assessed by medical history and physical examination and did not report orthostatic hypotension. None of the subjects used medication known to interfere with the cardiovascular system. Individuals with ankle-brachial pressure index <0.90, and/or abnormalities in a 12-lead resting ECG were excluded. The study has been carried out in accordance with the Declaration of Helsinki (2000) and was approved by the medical ethical committee of our institution. All subjects gave written informed consent.

Table 1. General characteristics of young and older men.

	Young men (n = 12)	Older men (n = 12)
Age (yrs)	24 ± 1	71 ± 1*
Height (cm)	182 ± 2	175 ± 3
Body mass (kg)	73 ± 3	78 ± 3
Systolic BP (mmHg)	124 ± 3	131 ± 4
Diastolic BP (mmHg)	74 ± 2	77 ± 2
MAP (mmHg)	90 ± 2	95 ± 2

Values represent mean±SE. BP, blood pressure. MAP, mean arterial blood pressure. *, significantly different from young men.

Experimental procedures and protocol

All subjects refrained from caffeine-containing food and beverages, vitamin C supplements, and alcohol for at least 12 hours prior to the experiment and refrained from heavy physical activity for at least 24 hours prior to the experiment. Room temperature was controlled at 23 ± 1 °C. After completing a health questionnaire, subjects were positioned comfortably on a tilt table in the supine position. The experimental procedures started after a supine resting period of at least 30 minutes.

First, baseline forearm and calf blood flow were measured for 5 minutes in the supine position. Subsequently, subjects were tilted manually, within 5 seconds, to a 5 minutes passive 30° HUT position in order to induce a significant cardiovascular response (12).

Measurements

Heart rate and arterial blood pressure were measured continuously using a non-invasive portable blood pressure device (Portapres, TNO, Amsterdam, the

Netherlands). A finger cuff was attached to the middle phalanx of the left third finger in order to measure finger arterial blood pressure. Finger arterial blood pressure measurements accurately reflect intra-arterial blood pressure changes during orthostatic stress (13). Data were collected during the experiment at a rate of 100 Hz. Mean arterial blood pressure (MAP) values were derived beat to beat and heart rate was the inverse of the interbeat interval.

Blood flow in the forearm and calf was measured using venous occlusion plethysmography. In the supine position, the right arm and leg were supported to ensure that they were positioned ~ 5 cm above heart level. A standard blood pressure cuff (10 cm width) was placed around the right upper arm and a 12 cm width occlusion cuff was placed just above the right knee. Mercury-in-silastic strain gauges (Hokanson, Inc, Bellevue, WA, USA) were placed at the widest girth of the right forearm and calf. Both cuffs were inflated simultaneously with a rapid cuff inflator (Hokanson, Inc, Bellevue, WA, USA), within 1 s, to 50 mmHg in the supine position (14) and to 65 mmHg in the 30° HUT position to correct for changes in hydrostatic pressure (15). The occlusion pressure was sustained for 7 seconds after which the cuffs were deflated instantaneously (for 9 seconds). Venous occlusion plethysmography is suggested to requisite an empty venous system to guarantee full venous compliance. Recently, it was shown that venous occlusion plethysmography measurements of calf blood flow during supine and up to 30° HUT correlates well ($r^2 = 0.86$, $p < 0.001$) with superficial femoral artery blood flow measured with Doppler ultrasound (16). This indicates that venous occlusion plethysmography during 30° HUT represents arterial inflow and is not significantly affected by venous compliance.

Data analysis

Blood flow (in $ml \cdot min^{-1} \cdot dl^{-1}$) was calculated from the slope of the volume change over a 4-seconds interval. To avoid artefacts, the initial first second was excluded from analysis. Vascular resistance was calculated as MAP divided by blood flow. For the calculation of vascular resistance, we assumed that venous pressure was ~ zero in the supine position. Furthermore, we assumed that hydrostatic pressure made an identical contribution to leg venous pressure as to leg arterial pressure during 30° HUT. Blood flow, vascular resistance, MAP and heart rate were averaged over the last two minutes of the supine and 30° HUT positions.

Statistical analysis

Results are expressed as mean ± SEM. A paired Student's t-test was used to determine the effect of 30° HUT in young and in older men and an unpaired Student's t-test was used to compare the changes upon 30° HUT between young and in older men. The level of statistical significance was set at $\alpha = 0.05$.

RESULTS

No differences in physical characteristics between young and older men were present, with exception of the significant age difference. (Table 1)

Central cardiovascular responses

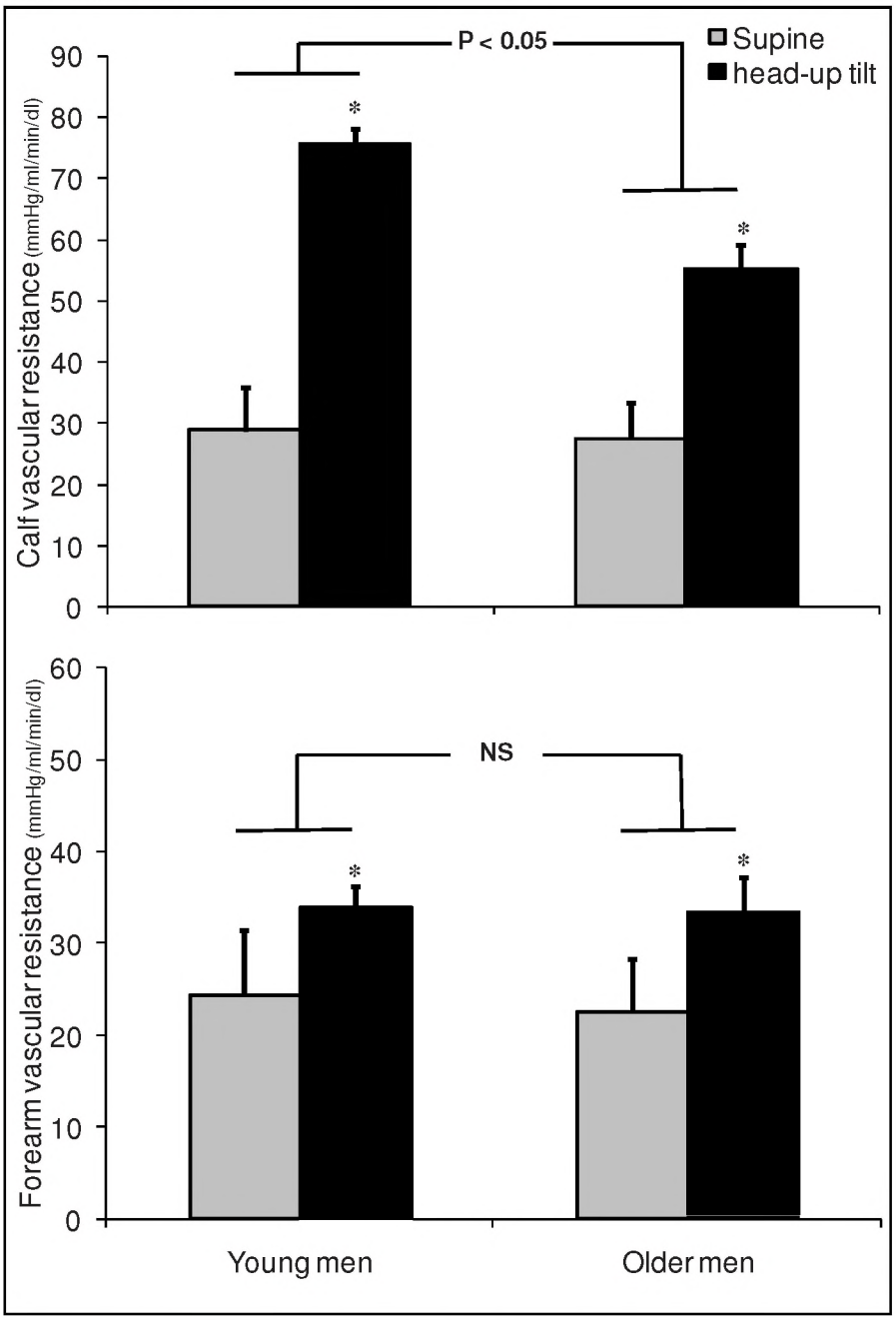
Young and older men demonstrated a significant increase in MAP during 30° HUT, which was significantly larger in young than in older men. During 30° HUT, heart rate increased significantly in young men only. (Table 2)

Table 2. Central and peripheral parameters during supine and 30° head-up tilt in young and older men.

	Supine	30° HUT	P-value
Young men (n = 12)			
MAP (mmHg)	85 ± 4	92 ± 4	< 0.001
Heart rate (bpm)	60 ± 2	66 ± 3	< 0.001
Calf BF ($ml \cdot min^{-1} \cdot dl^{-1}$)	3.3 ± 0.4	1.4 ± 0.1	< 0.001
Calf VR ($mmHg \cdot ml^{-1} \cdot min^{-1} \cdot dl^{-1}$)	28.8 ± 3.1	75.7 ± 8.7	< 0.001
Forearm BF ($ml \cdot min^{-1} \cdot dl^{-1}$)	4.9 ± 0.8	3.7 ± 0.6	< 0.001
Forearm VR ($mmHg \cdot ml^{-1} \cdot min^{-1} \cdot dl^{-1}$)	24.3 ± 4.2	33.7 ± 6.1	0.001
Older men (n = 12)			
MAP (mmHg)	87 ± 3	90 ± 3	0.010
Heart rate (bpm)	61 ± 4	63 ± 4	0.326
Calf BF ($ml \cdot min^{-1} \cdot dl^{-1}$)	3.4 ± 0.2	1.9 ± 0.2	< 0.001
Calf VR ($mmHg \cdot ml^{-1} \cdot min^{-1} \cdot dl^{-1}$)	27.5 ± 2.5	55.0 ± 7.9	< 0.001
Forearm BF ($ml \cdot min^{-1} \cdot dl^{-1}$)	4.8 ± 0.6	3.5 ± 0.5	< 0.001
Forearm VR ($mmHg \cdot ml^{-1} \cdot min^{-1} \cdot dl^{-1}$)	22.4 ± 2.9	33.2 ± 4.4	0.003

Values represent mean±SE. 30° HUT, 30° head-up tilt. MAP, mean arterial blood pressure. BF, blood flow. VR, vascular resistance.

Figure 1. 30° head-up tilt induced differences in calf and forearm vascular resistance in young and older men.



Values represent mean+SE. *, significantly different from supine, NS, not significantly different.

Peripheral cardiovascular responses

In young and older men, calf and forearm blood flow demonstrated a significant decrease during 30° HUT, which were not different between both groups. Calf and forearm vascular resistance increased significantly during 30° HUT in young and older men. The increase in forearm vascular resistance was not different between both groups. However, the increase in calf vascular resistance was affected by age. A significantly larger increase in calf vascular resistance was found in young (175 ± 30 %) compared with older men (96 ± 15 %). (Table 2 and Figure 1)

DISCUSSION

The major finding of the present study is that, in contrast to our hypothesis, an attenuated increase in calf vascular resistance during 30° HUT in older men is present compared with young men. The attenuated peripheral vasoconstriction in older men is present in the calf, but not in the forearm.

The lower limb vascular bed importantly contributes to the blood pressure regulation, especially during orthostatic stress (7). Hence, our results suggest that the age-related attenuated vasoconstriction in the calf vascular bed may be part of the explanation of orthostatic hypotension in older men.

We did not find a difference in baseline vascular tone between young and older men, which is in agreement with some studies (17-19), but in contrast with others (20,21). Studies which reported no difference in vascular tone used plethysmography to measure calf (17,18) or upper leg blood flow (19). Interestingly, studies that reported a difference in leg vascular tone (20,21), used Doppler ultrasound to measure whole leg blood flow. In contrast to plethysmography, Doppler does not correct for the differences in leg volume, which are likely to be present between these groups (22). In addition, Fu et al. (18) found no difference in calf blood flow between older and young men, although a higher sympathetic activity level was present in older men. This suggests that sympathetic activity can be increased, without an attenuated calf blood flow.

The augmented increase in total peripheral resistance during orthostatic challenges with advancing age is a well documented characteristic (8-11). In these studies, total peripheral resistance was studied, including the splanchnic vascular bed

that accounts for approximately one-third of the total peripheral resistance, we have assessed peripheral vascular responses of the calf and forearm that also accounts for approximately one-third of the total peripheral resistance. Minson et al. (11), demonstrated that older men increased their splanchnic vascular resistance to a greater extent than young men during 60° HUT, which was hypothesized to be a compensation for the attenuated cardiac output and diminished increase in vasoconstriction of the forearm vascular bed. In contrast, we found no differences in forearm vascular resistance in young and older men during 30° HUT. Interestingly, we demonstrated a lower ability to increase calf vascular resistance during 30° HUT in older compared with young men. Although a different tilt angle was used, this has no effect on leg blood flow (16). The attenuated increase in calf vascular resistance, therefore, may initiate or contribute, at least partly, to the orthostatic intolerance observed in older men.

We can only speculate about the possible mechanisms that explain the attenuated vasoconstriction response in the calf during 30° HUT in older men compared with young men. Recently, Smith et al. (23) demonstrated that leg postjunctional α-adrenergic vasoconstrictor responsiveness to noradrenalin is reduced with advancing age, and thereby can impede sympathetic control of vascular resistance and negatively influence blood pressure control to orthostatic challenges. In young men, a larger response in the calf to α-adrenergic receptor stimulation is present than in the forearm (24). Accordingly, it is possible that the lower limb is prone, especially with advancing age, to the reduction in α-adrenergic vasoconstrictor responsiveness and could explain the difference in tilt-induced calf vascular resistance increase between older and young men.

Another possible explanation for the tilt-induced attenuated increase in calf vascular resistance could be the attenuated vasoconstrictor reserve (25) in advancing age, possibly caused by an increase in basal sympathetic activity (18,26). In other words, older men cannot increase their calf vascular resistance during an orthostatic challenge to the same extent as young men, who have a larger vasoconstrictor reserve.
In addition, arterial and venous blood vessel stiffening may contribute to the orthostatic hypotension in older men (2,27). Blood vessel stiffening with advancing age is associated with decreased baroreflex sensitivity (2,28). The attenuated heart rate response during orthostatic challenges in older men (8,28), as present in our study, supports the decreased baroreflex sensitivity. On the contrary, blood vessel stiffening

could also blunt venous pooling and thereby limit the fall in venous return (27) during orthostatic challenges. However, since cardiac output in older men during orthostatic challenges is compromised (8-10), the vascular stiffening clearly is not sufficient enough to prevent venous pooling.

Another hypothesized mechanism is the decreased total blood volume in advancing age (29). During orthostatic challenges a translocation of blood into the compliant peripheral venous system occurs, leading to a decreased cardiac filling (7). In older men the lower total blood volume could lead to a relatively exaggerated decrease in cardiac filling during orthostatic challenges.

The objective of the present study was not to elucidate the mechanism causing the differences in tilt-induced responses between young and older men, for this additional research is needed. Nonetheless, the attenuated increase in calf vascular resistance during 30° HUT in older men seems to be part of the explanation of orthostatic hypotension in older men.

Clinical relevance

Individuals, who suffer from severe orthostatic hypotension, can promote venous return and thereby raise cardiac output by applying physical counter-manoeuvres (2,7,30). These physical counter-manoeuvres exist of leg crossing and/or leg muscle tensing, which are typically directed towards the lower limb vascular bed and have been demonstrated to be beneficial to counteract orthostatic hypotension. Based on our findings of an attenuated increase in calf vascular resistance in older men during 30° HUT, we suggest that applying these physical counter-manoeuvres could be helpful in counteracting orthostatic hypotension, especially with advancing age. Additional research is needed to evaluate the efficacy of these counter manoeuvres in the aging population.

Conclusion

In conclusion, the present study demonstrates that 30° head-up tilt leads to an attenuated increase in vascular resistance of the calf, but not of the forearm in older compared with young men. The lower ability to increase calf vascular resistance in older men during orthostatic challenges cannot explain the augmented total peripheral resistance during orthostatic challenges, but may be part of the explanation of orthostatic hypotension with advancing age.

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Lower vascular tone and larger plasma volume in Parkinson's disease with orthostatic hypotension

7

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Based on Movement Disorders, In Revision

ABSTRACT

Background: The pathophysiology of orthostatic hypotension in Parkinson's disease (PD) is incompletely understood. The primary focus has thus far been on central mediated vasoconstrictor mechanisms. Here, we test the role of two peripheral factors: (1) a reduced peripheral vasoconstriction (which may contribute because PD includes a generalized sympathetic denervation); and (2) an inadequate plasma volume (which may explain why plasma volume expansion can manage orthostatic hypotension in PD).

Methods: We included 11 PD patients with orthostatic hypotension (PD+OH), 14 PD patients without orthostatic hypotension (PD-OH) and 15 age-matched healthy controls. Leg blood flow was examined using duplex ultrasound during 60° head-up tilt. Leg vascular resistance was calculated as the arterial-venous pressure gradient divided by blood flow. In a subset of 9 PD+OH, 9 PD-OH and 8 controls, plasma volume was determined by indicator dilution method with radio-labelled albumin (^{125}I-HSA).

Results: The basal leg vascular resistance was significantly lower in PD+OH (0.7±0.3 mmHg/ml/min) compared to PD-OH (1.3±0.6 mmHg/ml/min) and controls (1.3±0.5 mmHg/ml/min). Leg vascular resistance increased significantly during 60° head-up tilt with no significant difference between the groups (35% in PD+OH; 47% in PD-OH and 57% in controls). Plasma volume was significantly larger in PD+OH (3869±265 ml) compared to PD-OH (3123±377 ml) and controls (3204±537 ml).

Conclusions: These results indicate that PD+OH have a lower basal leg vascular resistance in combination with a larger plasma volume compared with PD-OH and controls. Despite the increase in leg vascular resistance during 60° head-up tilt, PD+OH are unable to maintain their blood pressure.

INTRODUCTION

Symptomatic and asymptomatic orthostatic hypotension (OH) is present in up to 60% of patients with Parkinson's disease (PD) (1) and negatively correlated with quality of life (2). In elderly, OH is associated with cardiovascular morbidity and mortality (3-5). The pathophysiology of OH in PD remains incompletely understood. Until now, the focus has been primarily on impairment of central mediated vasoconstrictor mechanisms. PD patients have a reduced baroreflex cardio-vagal function (6), denervation of the heart (6,7), low noradrenaline levels and no increment in noradrenaline levels during orthostatic challenges (6,8). However, baroreflex failure and cardiac sympathetic denervation contribute to but cannot fully explain OH in PD (6,9).

During orthostatic challenges peripheral vasoconstriction contributes to maintain blood pressure via a central vasoconstrictor mechanism, i.e. baroreflex, and local vasoconstrictor mechanisms, such as the veno-arteriolar axon reflex (10) and the myogenic response (11). Since in PD a generalized rather than a central sympathetic denervation is present (12), central and local mediated peripheral vasoconstriction might be affected and, thereby, might play an important role in the pathophysiology of OH in PD.

Furthermore, a lower plasma volume may be another contributing factor to OH in PD. This assumption is based on the important role of the sympathetic nervous system in volume homeostasis via renal innervation and the renin-angiotensin system (13). In addition, a relation is present between baroreflex mediated vascular control (14), time to presyncope (15) and blood pressure during orthostatic challenges in autonomic failure (16). In addition, plasma volume expansion is considered an effective therapy in PD patients with OH (17,18). However, data on possible baseline differences in plasma volume in PD are lacking.

The aim of this study was to assess peripheral vascular responses in PD patients with and without OH, as well as in controls. For this purpose, we measured leg vascular resistance at baseline and during 60° head-up tilt. In addition, we aimed to measure plasma volume in these three groups. We hypothesized that baseline leg vascular resistance, the leg vascular resistance increase during 60° head-up tilt and plasma volume are lower in PD patients with OH compared with PD patients without OH and controls.

METHODS

Subjects

25 PD patients and 15 age- and gender matched controls participated. Patients had idiopathic PD (19) diagnosed by an experienced neurologist (R.A.J.E. or B.R.B.) and disease severity (Hoehn and Yahr stages (20)) and severity of motor symptoms (Unified Parkinson Disease Rating Scale (UPDRS part III (21)) were assessed. PD patients were assigned to two groups according to the presence of OH. OH was defined as a decrease of ≥20 mmHg in systolic or ≥10 mmHg in diastolic blood pressure during the first 3 minutes of active standing or during a passive 60° head-up tilt (22). 11 PD patients fulfilled the consensus criteria of OH (PD+OH), leaving 14 PD patients without OH (PD-OH) (Table 1). The two patient groups showed no significant differences for various important baseline variables, including disease duration, disease severity and levodopa equivalent dose (Table 1) (23,24). None of the controls fulfilled the OH criteria. Exclusion criteria were smoking, cardiovascular disease, diabetes and hypertension. None of the subjects used medication known to relevantly interfere with vascular function, except for Parkinson medication which was continued during the study. In a randomly selected subgroup, of the original study group, we determined plasma volume (Table 2).

The study was performed in accordance with the Declaration of Helsinki and approved by the medical ethical committee of our institution. All subjects gave written informed consent.

Experimental procedures and protocol

Prior to the head-up tilt experiment and the plasma volume measurement, all subjects refrained from caffeine-containing beverages, vitamin C supplements and alcohol for >12 hours and from heavy physical activity for >24 hours and had been fasting for >2 hours. All head-up tilt experiments and plasma volume measurements were performed in the morning in a quiet, temperature-controlled room (23±1°C).

Subjects laid down on a manually driven tilt-table with footboard. The subjects supported their body weight during the 60° head-up tilt by standing on the left leg, allowing the right leg to be relaxed for blood flow measurements. After a supine resting period of at least 30 minutes, subjects were tilted manually, within 5 seconds,

to a passive 60° head-up tilt position for a 10-minute period. In the subgroup plasma volume was determined on a separate occasion.

Table 1. Subject characteristics.

	Controls (n=15)	PD-OH (n=14)	PD+OH (n=11)
Gender (m/f)	9/6	6/8	10/1*
Age (yrs)	64±8	61±8	65±9
Height (cm)	175±8	172±10	179±9
Body mass (kg)	77±9	75±14	83±11
Systolic BP (mmHg)	126±15	130±13	131±12
Diastolic BP (mmHg)	81±7	81±9	80±6
Disease duration (yrs)		7±5	10±10
H & Y stage (median(range))		2(1-3)	2(1.5-4)
UPDRS III (median(range))		23(7-40)	26(13-63)
Dopaminergic medication			
Levodopa (mg/day)		581±230 (n=8)	564±253 (n=7)
Ergoline dopamine agonist (mg/day)		3 (n=1)	
Non-ergoline dopamine agonist (mg/day)		5±5 (n=6)	6±8 (n=9)
Levodopa equivalent dose (mg/day)		500±316 (n=11)	615±286 (n=8)

Values represent mean±SD. PD-OH, Parkinson disease without orthostatic hypotension. PD+OH, Parkinson disease with orthostatic hypotension. BP, blood pressure. H & Y stage, Hoehn & Yahr stage (range: 1-5). UPDRS III, Unified Parkinson Disease Rating Scale part III (range: 0-104).*significantly different from PD-OH and controls.

Table 2. Subject characteristics of subgroup for plasma volume determinations.

Characteristics	Controls (n=8)	PD-OH (n=9)	PD+OH (n=9)
Gender (m/f)	6/2	6/3	8/1
Age (yrs)	66±7	60±7	62±7
MAP (mmHg)	97±11	97±9	94±5
Body mass index (kg/m^2)	25±2	26±4	26±3
Lean body mass (LBM) (kg)	60±6	58±8	62±3
Disease duration (yrs)		9±8	7±5
H & Y stage (median(range))		2(1-2.5)	2(2-2.5)
UPDRS III (median(range))		26(12-40)	26.5(15-39)
Dopaminergic medication			
Levodopa (mg/day)		533±264 (n=6)	542±489 (n=7)
Ergoline dopamine agonist (mg/day)		3 (n=1)	
Non-ergoline dopamine agonist (mg/day)		7±7 (n=3)	11±9 (n=4)
Levodopa equivalent dose (mg/day)		530±334	702±516

Values represent mean±SD. PD-OH, Parkinson disease without orthostatic hypotension. PD+OH, Parkinson disease with orthostatic hypotension. BP, blood pressure. H & Y stage, Hoehn & Yahr stage (range: 1-5). UPDRS III, Unified Parkinson Disease Rating Scale part III (range: 0-104).*significantly different from PD-OH and controls.

Measurements

Blood pressure was measured continuously using a non-invasive blood pressure device (Nexfin, BMEYE, The Netherlands). A finger cuff was attached to the middle phalanx of the left third finger to measure finger arterial blood pressure, which accurately reflects intra-arterial blood pressure changes (25). A build-in heart reference system was in operation to correct for hydrostatic influences. Mean arterial blood pressure (MAP) was derived beat to beat and heart rate was the inverse of the interbeat interval.

Upper leg blood flow during the last minute of supine rest and 60° head-up tilt was measured using duplex ultrasound. Mean red blood cell velocity (Vmean) and diameter of the right superficial femoral artery, ~2 cm distal of the bifurcation, were measured with a duplex ultrasound device (ARTLAB system, Pie Medical, The Netherlands; WAKI, Atys Medical, France). Vmean was calculated as the average of 20 ultrasound waveforms and diameter was calculated as the average of 6 consecutive mean diameters. Leg blood flow was calculated as $(\pi \cdot r^2 \cdot \text{Vmean}) \cdot 60$ ($r = \frac{1}{2} \cdot$ diameter of the superficial femoral artery).

Calf venous pooling was determined by measuring the calf volume change using plethysmography. Mercury-in-silastic strain gauges were placed around the thickest part of the right calf and connected to a plethysmograph (Hokanson Inc, USA). To avoid any direct contact with the tilt-table, the calf was supported by small cushions. The difference in calf volume between 60° head-up tilt and supine was determined and expressed as percentage increase.

Venous blood samples during supine rest and 60° head-up tilt were taken to determine plasma noradrenaline levels as described previously (26).

Plasma volume was determined by indicator dilution method using radio-labelled albumin (^{125}I-HSA) (27). A known quantity of ^{125}I-HSA was injected into an antecubital vein in supine position. Subsequently, venous blood samples from the contra-lateral antecubital vein were drawn after 10, 20, 30 and 40 minutes.

Data analysis

Leg vascular resistance was calculated as the arterial–venous pressure gradient divided by leg blood flow. Supine venous pressure was set at 9 mmHg and during 60° head-up tilt the arterial-venous pressure gradient was replaced by MAP, since hydrostatic

pressure makes an identical contribution to leg venous as well as leg arterial pressure (28).

Plasma volume was determined by dividing the total injected radioactivity by the virtual volume-specific radioactivity at time 0, which corresponds with the injection of ^{125}I-HSA (27). Measured plasma volume was expressed according to the calculated lean body mass (29) and a predicted plasma volume was calculated (30).

Statistical analysis

Data are presented as mean±SD, unless otherwise stated. The level of statistical significance was set at α=0.05. Unpaired t-tests were used to assess differences in predicted and measured plasma volume within the groups. ANOVAs were used to assess differences in subject characteristics, baseline supine values, 60° head-up tilt values and plasma volume. Repeated measures ANOVAs were used to assess the effect of 60° head-up tilt. Bonferroni's correction was used to correct for multiple comparisons. Post-hoc t-tests were performed when the ANOVA reported a significant effect.

Table 3. Central and peripheral hemodynamic during supine and 60° head-up tilt.

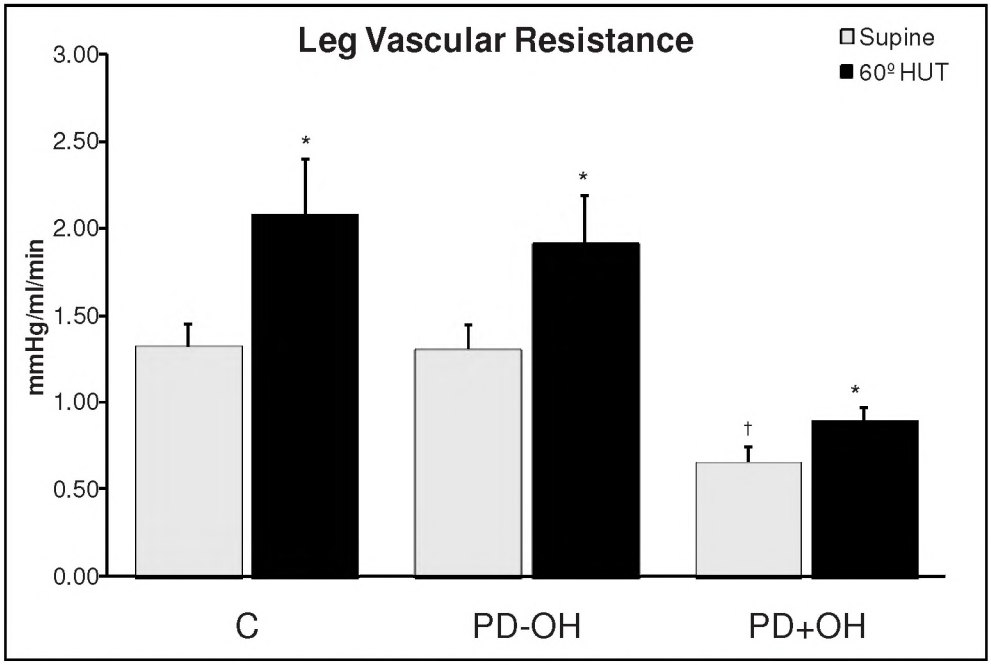
	Controls *(n=15)*		**PD-OH** *(n=14)*		**PD+OH** *(n=11)*		**ANOVA**	
	Supine	*60°HUT*	*Supine*	*60°HUT*	*Supine*	*60°HUT*	P_{time}	$P_{time*group}$
SBP (mmHg)	129±22	125±24	129±10	131±11	130±11	100±18*†	<0.01	<0.01
DBP (mmHg)	83±7	85±9	82±8	87±11	84±4	78±10	0.68	<0.01
MAP (mmHg)	99±11	98±12	97±8	101±9	99±6	86±12*†	<0.01	<0.01
Heart rate (bpm)	58±7	71±13	68±7*‡	83±9*	60±7	74±10	<0.01	0.64
SFA (mm)	7.7±1.2	7.5±1.2	6.7±0.8*‡	6.6±0.7*‡	8.2±0.7	8.0±0.7	<0.01	0.55
LBF (ml/min)	85±32	64±36	88±35	77±62	184±76*†	111±66	<0.01	0.01
LVR (mmHg/ml/min)	1.32±0.51	2.07±1.25	1.30±0.58	1.91±1.10	0.66±0.34*†	0.89±0.28*†	<0.01	0.22
NA (nmol/l)	1.97±0.70	2.60±0.87	1.94±0.89	2.25±1.17	1.79±0.56	1.76±0.61	<0.01	0.02
Calf volume change (%)		3.0±0.6		2.7±0.8		2.1± 0.6*		

Values represent mean±SD. PD-OH, Parkinson disease without orthostatic hypotension. PD+OH, Parkinson disease with orthostatic hypotension. HUT, head-up tilt. SBP, systolic blood pressure. DBP, diastolic blood pressure. MAP, mean arterial blood pressure. SFA, superficial femoral artery diameter. LBF, leg blood flow. LVR, leg vascular resistance. NA, noradrenaline. *, significantly different from controls. †, significantly different from PD-OH. ‡, significantly different from PD+OH

RESULTS

In the PD+OH group, baseline leg blood flow was significantly higher and leg vascular resistance was significantly lower compared with PD-OH ($p<0.01$ for both parameters) and controls ($p<0.01$ for both parameters) (Table 3, Figure 1). The diameter of the superficial femoral artery in PD+OH ($p<0.01$) and controls ($p=0.02$) was significantly larger compared with PD-OH (Table 3). Heart rate in PD-OH was significantly higher compared to PD+OH ($p=0.02$) and controls ($p<0.01$) (Table 3). No differences in baseline systolic and diastolic blood pressure, MAP or noradrenaline levels were observed (Table 3).

Figure 1. Leg vascular resistance in supine and 60° head-up tilt position in controls, Parkinson's disease patients without and with orthostatic hypotension.



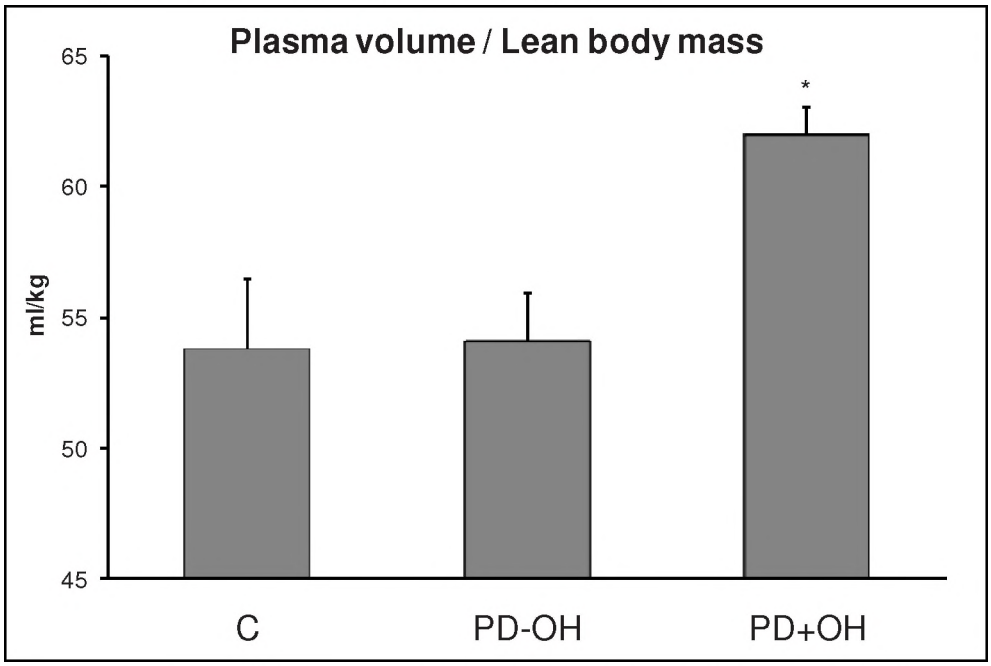
Values represent mean+SE. C, controls (n=15). PD-OH, Parkinson's disease without orthostatic hypotension (n=14). PD+OH, Parkinson's disease with orthostatic hypotension (n=11). HUT, head-up tilt. *, significantly different from supine position. †, significantly different from C and PD-OH.

During 60° head-up tilt, systolic blood pressure and MAP significantly decreased in PD+OH (Table 3). Heart rate significantly increased in all groups with a similar magnitude (Table 3). Leg blood flow decreased significantly in all groups during 60°

head-up tilt, with a higher decrease in PD+OH (40%) compared with PD-OH (13%) and controls (25%) (Table 3). Leg vascular resistance significantly increased during 60° head-up tilt in PD+OH (35%), PD-OH (47%) and controls (57%), with no significant difference between the groups (Table 3, Figure 1). Leg vascular resistance during 60° head-up tilt in PD+OH was significantly lower compared with PD-OH ($p<0.01$) and controls ($p=0.01$) (Table 3, Figure 1). The superficial femoral artery diameter significantly decreased during 60° head-up tilt in PD+OH and controls but not in PD-OH (Table 3). Noradrenaline levels significantly increased during 60° head-up tilt in controls and PD-OH but not in PD+OH (Table 3).

Calf volume increased during 60° head-up tilt in all groups, with a significant lower calf volume increase in PD+OH compared with controls (Table 3).

Figure 2. Plasma volume expressed per lean body mass in controls, Parkinson's disease patients without and with orthostatic hypotension.



Values represent mean+SE. C, controls (n=8). PD-OH, Parkinson's disease without orthostatic hypotension (n=9). PD+OH, Parkinson's disease with orthostatic hypotension (n=9). *, significantly different from PD-OH and C.

Plasma volume was significantly larger in PD+OH compared with PD-OH ($p<0.01$) and controls ($p<0.01$) (Table 4). In PD+OH the measured plasma volume was

significantly larger ($p<0.01$) compared with predicted (Table 4). Plasma volume corrected for lean body mass was significantly larger in PD+OH compared with PD-OH ($p<0.01$) and controls ($p<0.01$) (Table 4, Figure 2).

Table 4. Plasma volume.

	Controls (n=8)	PD-OH (n=9)	PD+OH (n=9)
Predicted plasma volume (ml)	3003±295	2957±372	3190±100
Plasma volume (ml)	3204±537	3123±377	3869±265*†
Plasma volume / LBM (ml/kg)	53.8±7.6	54.1±5.6	62.0±3.1*
LVR (mmHg/ml/min)	1.17±0.40	1.28±0.65	0.88±0.31

Values represent mean±SD. PD-OH, Parkinson disease without orthostatic hypotension. PD+OH, Parkinson disease with orthostatic hypotension. LVR, baseline leg vascular resistance. *, significantly different from controls and PD-OH. †, significantly different from predicted plasma volume.

DISCUSSION

The major findings of this study are that PD+OH have a lower basal leg vascular resistance, in combination with a larger plasma volume, as compared with both PD-OH and controls. The leg vascular resistance increase during 60° head-up tilt was not different between the three groups. Despite the leg vascular resistance increase during 60° head-up tilt PD+OH are unable to maintain their blood pressure.

The present study is the first to demonstrate a lower basal leg vascular resistance in PD+OH compared with PD-OH and controls. This novel finding is in agreement with our hypothesis and in line with the accepted pathophysiology of OH in PD. Since PD is associated with a generalized rather than a central sympathetic denervation (12), an attenuated sympathetic activity may explain the lower leg vascular resistance in PD+OH. Muscle sympathetic nerve activity (MSNA) in PD decreases with age, disease duration and severity (31). This might indicate that generalized sympathetic denervation is more pronounced in PD+OH than in PD-OH. However, denervation of sympathetic fibers to the heart has been shown to exist not only in PD+OH but also in PD-OH and even in early PD (6,7,32). A second explanation could be the lower noradrenaline levels in PD+OH. We did not find a significant difference in basal noradrenaline levels between the groups, in contrast to other studies (1,6,8,12), although noradrenaline in our study tended to be lower in PD+OH. The lack of a noradrenaline difference could be due to larger standard deviations of noradrenaline

levels in our PD patients who are treated with levodopa (9), Although noradrenaline is a potent vasoconstrictor, vascular tone is regulated by a combination of several vasoconstrictors and dilators. The effect of lower noradrenaline levels in PD+OH on vascular tone and leg vascular resistance is unknown. A decrease in α-adrenoceptors or in their sensitivity to noradrenaline could be a third explanation, especially in combination with lower noradrenaline levels. However, in PD+OH there is evidence for α-adrenoceptor supersensitivity caused by sympathetic denervation (33). The explanation for the lower basal leg vascular resistance in PD+OH is, therefore, most likely an attenuated sympathetic activity to the legs.

In contrast to our hypothesis leg vascular resistance increased during 60° head-up tilt in PD+OH, and to a similar extent as in PD-OH and controls. During orthostatic challenges an immediate drop in blood pressure is sensed by baroreceptors. This increases heart rate, cardiac contractility and peripheral vascular resistance via an increase in sympathetic outflow and a decrease in vagal-nerve activity (34,35). One previous study measured local vascular resistance of the anterior tibial muscle and subcutaneous tissue of the calf in PD+OH during 45° head-up tilt using the 133-Xenon washout method, but did not find a significant increase (36). The smaller experimental vascular bed, lower tilt angle and small number of subjects could explain the difference with our study. The leg vascular resistance increase during 60° head-up tilt in PD+OH in our study might be explained by spared sympathetic fibers. However, noradrenaline levels in PD+OH did not increase during 60° head-up tilt, in concordance with previous studies (6,8). An increase in sympathetic activity is, therefore, unlikely to explain the leg vascular resistance increase in PD+OH. Another explanation might be the local vasoconstrictor mechanisms involved in maintaining blood pressure during orthostatic challenges, i.e. the veno-arteriolar axon reflex (VAR) and the myogenic response. Preserved local vasoconstriction mechanisms have been previously demonstrated in a small number of PD+OH and allocated to the VAR (36). The VAR is a local axon reflex triggered by an >25 mmHg increase in venous pressure which results in constriction of the corresponding arteriole and assumed to be α-adrenergic mediated (10). Since in PD+OH there is no increase in noradrenaline levels, it is unlikely that the VAR contributes to the leg vascular resistance increase during 60° head-up tilt. More likely, the myogenic response is responsible for the observed leg vascular resistance increase in PD+OH. The myogenic response is a smooth muscle vasoconstrictor response to increments in transmural pressure

independent of the sympathetic nervous system (11). The myogenic response is thought to play a major role during orthostatic challenges in autonomic failure (11). The myogenic response can, therefore, at least partly explain the leg vascular resistance increase in PD+OH during 60° head-up tilt whether or not accompanied by a partly spared central mediated α-adrenergic vasoconstriction.

Although the increase in leg vascular resistance during 60° head-up tilt was not significantly lower in PD+OH compared to PD-OH and controls, the leg vasoconstriction in PD+OH is insufficient to counteract the decrease in blood pressure during 60° head-up tilt. The compensatory local vasoconstrictor mechanisms are unable to increase leg vascular resistance in PD+OH during 60° head-up tilt to a satisfactory level that can prevent the development of OH. A lack of appropriate leg vasoconstriction might, therefore, play a role in the pathophysiology of OH in PD. Especially in combination with the lower basal leg vascular resistance in PD+OH.

A larger calf volume increase is associated with a larger decrease in venous return and, thereby, can contribute to OH (34). In contrast, during 60° head-up the calf volume increase was lower in PD+OH compared to controls and tended to be lower compared to PD-OH. Excessive venous pooling of the calf does, therefore, not contribute to OH in PD. However, venous pooling in other vascular beds, especially the splanchnic vascular bed, might still play a role in a decreased venous return in PD+OH.

Besides central and local mediated vasoconstrictor mechanisms, an adequate plasma volume is essential for orthostatic tolerance (35,37). In contrast to our hypothesis, the plasma volume in PD+OH was larger compared to PD-OH and controls. The larger plasma volume in PD+OH could be a compensation for the lower leg vascular resistance. As a result of the lower leg vascular resistance, vascular capacity is increased and will result in a relative low plasma volume (37) which could lead to a reduced renal arterial pressure. Consequently, renin will be released and more angiotensin II will be formed leading to aldosterone release, and thereby increasing plasma volume (38). However, plasma renin concentration in PD seems to be lower than in controls and PD patients show an inadequate rise in plasma renin during head-up tilt (39). Unfortunately, we do not know whether the renin-angiotensin-aldosterone system in PD+OH is activated. Vasopressin or antidiuretic hormone are unlikely to play a role, since plasma levels in a small group of PD+OH were comparable to controls (40) and vasopressin treatment had no effect (41).

Despite the larger plasma volume, PD+OH are not able to maintain their blood pressure in an upright position. Possibly, they still have a relative low plasma volume as a result of the lower basal leg vascular resistance (37). We controlled for other causes of plasma volume increase (38,42,43), e.g. none of the subjects had a high dietary salt intake, slept in a head-up tilt position, used volume expanders and there were no differences in physical activity between the groups. Another, more technical, explanation could be related to the plasma volume measurement. Considerable endothelial damage could cause leakage of ^{125}I-HSA into the extravascular space which will cause an overestimation of plasma volume. In PD endothelial function and possible damage is unknown. Since the renal endothelium is especially vulnerable, proteinuria should be one of the first symptoms. None of the PD+OH had known proteinuria and, therefore, the dilution method with ^{125}I-HSA is considered to be the golden standard (27).

Limitations

The PD+OH group included more men, and patients tended to be more severely affected compared with PD-OH and controls. They also used a higher levodopa equivalent dose, although the difference with PD-OH was not significant. OH in PD is associated with gender, age, disease duration and severity (44), however this does not explain the demonstrated lower basal leg vascular resistance in combination with the larger plasma volume.

We measured plasma volume in a subgroup for practical reasons. We randomly selected the subjects from the original study group in order to avoid bias. The results of the plasma volume determination are convincing, even in our relatively small sample size. We do not believe that a larger sample size would change our results.

Clinical Perspective

Despite several published recommendations on the treatment of OH in PD, they are scarcely evidenced based (17,18). Several non-pharmacological interventions have presumed efficacy by increasing venous return or plasma volume, i.e. elastic stockings, physical counter manoeuvres, salt and fluid intake, sleeping in the head-up position or increase physical activity (17,18). Fludrocortisone (9α-fluorohydrocortisone) and midodrine (α-adrenoceptor agonist) are most commonly used in the pharmacological management of OH in PD (17,18). The results of the present study, i.e. a lower basal

leg vascular resistance with low noradrenaline levels and a larger plasma volume in PD+OH, support the use of midodrine as a vasoconstrictive agent and, at the same time, question the use of fludrocortisone as a volume expanding agent. More evidence is, therefore, needed in non- and pharmacological management of OH in PD in order to optimally manage OH in PD.

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Leg vasoconstriction during head-up tilt in patients with autonomic failure is not abolished

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ABSTRACT

Maintaining blood pressure during orthostatic challenges is primarily achieved by baroreceptor mediated activation of the sympathetic nervous system, which can be divided into a preganglionic and postganglionic part. Despite their preganglionic autonomic failure, spinal cord-injured individuals demonstrate a preserved peripheral vasoconstriction during orthostatic challenges. Whether this also applies to patients with postganglionic autonomic failure is unknown. Therefore, we assessed leg vasoconstriction during 60° head-up tilt in five patients with pure autonomic failure (PAF) and in two patients with autonomic failure due to dopamine-ß-hydroxylase (DBH) deficiency. Ten healthy subjects served as controls. Leg blood flow was measured using duplex ultrasound in the right superficial femoral artery. Leg vascular resistance was calculated as the arterial-venous pressure gradient divided by blood flow. DBH deficiency patients were tested off and on the noradrenalin pro-drug L-DOPS. During 60° head-up tilt, leg vascular resistance increased significantly in PAF (0.40±0.38 (+30%) mmHg/ml/min). The increase in leg vascular resistance was not significantly different from controls (0.88±1.04 (+72%) mmHg/ml/min). In DBH deficiency leg vascular resistance increased by 0.49±0.01 (+153%) and 1.52±1.47 (+234%) mmHg/ml/min off and on L-DOPS, respectively. Despite the increase in leg vascular resistance, orthostatic hypotension was present in PAF and DBH deficient patients. Our results demonstrate that leg vasoconstriction during orthostatic challenges in patients with PAF or DBH deficiency is not abolished. This indicates that the sympathetic nervous system is not the sole or pivotal mechanism inducing leg vasoconstriction during orthostatic challenges. Additional vasoconstrictor mechanisms may compensate for the loss in sympathetic nervous system control.

INTRODUCTION

The upright posture in humans leads to a gravitational displacement of blood into the dependent vasculature of the splanchnic area and lower limbs, leading to a decrease in venous return to the heart and an immediate drop in blood pressure (1,2). The decrease in blood pressure will unload baroreceptors resulting in a decreased vagal-nerve activity and increased sympathetic outflow (1,2). The activation of the sympathetic nervous system will increase heart rate, cardiac contractility and peripheral vascular resistance (1,2).

The sympathetic nervous system can be anatomically and functionally divided in a preganglionic and postganglionic part (1,3). Despite preganglionic autonomic failure, spinal cord-injured individuals demonstrate an increase in leg vascular resistance during orthostatic challenges (4-7). These results suggest that the sympathetic nervous system is not obligatory for peripheral vasoconstriction during orthostatic challenges. Whether this also applies to patients with postganglionic autonomic failure is unknown. We, therefore, assessed leg vasoconstriction during an orthostatic challenge in two distinctive patient groups with autonomic disorders who demonstrate selective failure of the postganglionic part of the sympathetic nervous system.

Table 1. Pathophysiological classification.

	PAF	DBH
Sympathetic nerves	Degenerative	Intact
Baseline noradrenaline	Low	Absent
Noradrenaline response during tilt	Low	Absent
Response to noradrenaline	Increased	Increased

PAF, pure autonomic failure. DBH, dopamine-ß-hydroxylase deficiency. SNS, sympathetic nervous system.

For this purpose, we measured leg vascular resistance at baseline and during 60° head-up tilt in patients with pure autonomic failure (PAF) and dopamine-ß-hydroxylase (DBH) deficiency as well as a group of healthy controls. PAF represents a chronic primary autonomic disorder with a postganglionic lesion with sympathetic denervation (Table 1) (8,9). DBH deficiency is an extremely rare hereditary autonomic disorder characterized by a complete absence of noradrenaline with an intact sympathetic nervous system (Table 1) (10). We hypothesized that leg

vasoconstriction will be present in patients with PAF and DBH deficiency, which supports the idea that the sympathetic nervous system is not obligatory for leg vasoconstriction during orthostatic challenges.

METHODS

Subjects

Seven patients with chronic autonomic failure, including 5 PAF and 2 DBH deficient patients, and 10 age-matched healthy controls (7 males and 3 females) participated in this study (Table 2). PAF is characterised by reduced levels of noradrenaline and widespread autonomic failure with no other neurological features present (11) (Table 1) and was diagnosed by an experienced internist (J.D. or J.W.M.L.). Diagnosis of DBH deficiency was confirmed by a complete absence of plasma and urine noradrenaline and adrenaline (Table 1), increased dopamine levels, a significant improvement of clinical symptoms of autonomic failure upon L-threo-dihydroxyphenylserine (L-DOPS, droxidopa) treatment and verification of the genetic disorder (10,12). None of the subjects smoked. All PAF patients continued their medication (Table 2) and four of them were put on extra sodium intake. DBH deficiency patients were measured before and 2 weeks after cessation of their daily L-DOPS treatment, which was confirmed by undetectable plasma and urine noradrenaline levels. Autonomic failure in PAF patients was confirmed by Valsalva manoeuvre testing.

This study was performed in accordance with the Declaration of Helsinki and approved by the medical ethical committee of our institution. All subjects gave written informed consent.

Experimental procedures and protocol

All subjects refrained from caffeine-containing food and beverages, vitamin C supplements, nicotine and alcohol for >12 hours and from heavy physical activity for >24 hours prior to the experiment. Subjects had been fasting for >2 hours before the experiment. All experiments were performed in the morning in a quiet, temperature-controlled (23±1 °C) room.

Subjects rested in the supine position on a manually driven tilt-table with footboard. After a supine resting period of at least 30 minutes, subjects were tilted

manually, within 5 seconds, to a passive 60° HUT position for a 10-minute period. The subjects supported their body weight during the 60° head-up tilt (HUT) by standing on the left leg, allowing the non-weight bearing right leg to be relaxed for blood flow measurements.

Table 2. Subject and group characteristics.

Subject	Gender	Age (yrs)	Height (cm)	Weight (kg)	SBP (mmHg)	DBP (mmHg)	HR (bpm)	Drugs
DBH1	f	26	159	60	136	72	52	L-DOPS
DBH2	f	41	167	55	120	90	56	L-DOPS
DBH (n=2)		**34±11**	**163±6**	**58±4**	**128±11**	**81±3**	**54±3**	
PAF1	f	69	154	52	140	76	85	1,2
PAF2	m	75	184	61	120	86	78	
PAF3	f	76	172	60	144	86	68	1,2
PAF4	f	64	167	74	192	98	68	1,2,
PAF5	m	67	172	54	180	90	68	2
PAF (n=5)		**70±5**	**170±11**	**60±9***	**155±30***	**87±8**	**73±8**	
C (n=8)		**69±4**	**177±9**	**80±9**	**128±18**	**81±8**	**60±8**	

Values represent individual values and mean±SD. DBH, dopamine-ß-hydroxylase deficiency. PAF, pure autonomic failure. C, controls. SBP, systolic blood pressure. DBP, diastolic blood pressure. HR, heart rate. L-DOPS, L-threo-dihydroxyphenylserine. 1, midodrine. 2, fludrocortisone. *, significantly different from controls. Blood pressure and heart rate values for DBH are after a 14 days interruption of L-DOPS treatment.

Measurements

Blood pressure was measured continuously using a non-invasive blood pressure device (Nexfin, BMEYE). A finger cuff was attached to the middle phalanx of the right third finger to measure finger arterial blood pressure, which accurately reflects intra-arterial blood pressure changes (13). A built-in heart reference system was in operation to correct for hydrostatic influences. Mean arterial blood pressure (MAP) was derived beat to beat and heart rate was the inverse of the interbeat interval. Stroke volume and systemic vascular resistance was determined by a three-element model of arterial input impedance using Modelflow (14). Cardiac output was calculated as stroke volume times heart rate.

Superficial femoral arterial blood flow during supine rest and 60° HUT was measured using duplex ultrasound. Mean red blood cell velocity (Vmean) and systolic and diastolic diameter of the right superficial femoral artery, ~2 cm distal of the bifurcation, were measured with a duplex ultrasound device (Picus, ESAOTE; WAKI,

Atys Medical). Vmean was calculated as the average of 20 consecutive Doppler waveforms. Automated software was used for operator-independent analyses of waveforms (Matlab 6.1; Mathworks). For diameter measurements the average of six consecutive mean diameters was obtained. Real-time automated analyses were performed using the ARTLAB system (Pie Medical). Leg blood flow was calculated with the following formula: $(\pi \cdot r^2 \cdot Vmean) \cdot 60$ ($r = \frac{1}{2} \cdot$ diameter of the superficial femoral artery).

Data analysis

Leg vascular resistance was calculated as the arterial-venous pressure gradient (P_a-P_v) divided by blood flow. Supine venous pressure was set at 9 mmHg and during 60° HUT the arterial-venous pressure gradient was replaced by MAP, since hydrostatic pressure makes an identical contribution to leg venous as well as leg arterial pressure (15).

Statistical analysis

Statistical analyses were performed using SPSS 16.0 (SPSS) software. Data are presented as mean±SD, unless otherwise stated. The level of statistical significance was set at $\alpha<0.05$. Differences in baseline parameters between PAF and controls were assessed using independent t-tests. Repeated measures ANOVAs were used to assess differences in the effect of 60° HUT on leg vascular resistance between PAF and controls. *Post hoc* t-tests were performed when the ANOVA reported a significant effect. Bonferroni correction was used to correct for multiple comparisons. Baseline values and the effect of 60° HUT in DBH deficiency patients were not statistically analyzed.

RESULTS

Baseline leg vascular resistance, leg blood flow and superficial femoral artery diameter were not significantly different between PAF and controls, whereas MAP and heart rate were significantly higher in PAF compared to controls ($p=0.01$ and $p<0.001$, respectively) (Table 3). In DBH deficiency patients baseline supine leg vascular resistance was lower compared to controls (Table 3). Baseline supine MAP and heart rate were comparable between DBH deficiency and controls (Table 3). Although leg

vascular resistance increased during L-DOPS treatment, leg vascular resistance remained lower in DBH deficiency compared to controls (Table 3).

Table 3. Basal and 60° head-up tilt systemic and peripheral cardiovascular parameters.

Subject	MAP (mmHg)		Heart rate (bpm)		LBF (ml/min)		LVR (mmHg/ml/min)		SFA
	Basal	*60° HUT*	*Basal*	*60° HUT*	*Basal*	*60° HUT*	*Basal*	*60° HUT*	*(mm)*
DBH1	99	81	51	58	332	109	0.27	0.77	6.8
on L-DOPS	95	82	47	55	140	35	0.62	3.17	6.1
DBH2	86	52	56	78	199	59	0.39	0.87	5.3
on L-DOPS	101	87	68	69	111	67	0.83	1.31	5.7
PAF1	97	86	85	89	55	45	1.66	1.77	9.0
PAF2	119	98	79	94	72	54	1.53	1.87	6.2
PAF3	133	54	63	69	80	22	1.61	2.66	6.5
PAF4	150	64	70	63	180	61	0.79	1.06	9.5
PAF5	121	80	69	75	104	62	1.08	1.32	9.0
PAF (n=5)	**124±19***	**76±18*†**	**73±9***	**78±13**	**98±49**	**49±16†**	**1.33±0.38**	**1.74±0.61†**	**8.0±1.6**
C (n=8)	**101±12**	**101±13**	**59±8**	**71±12†**	**96±33**	**63±30†**	**1.17±0.39**	**2.04±1.23†**	**8.0±1.2**
	Time	*Interact*	*Time*	*Interact*	*Time*	*Interact*	*Time*	*Interact*	
ANOVA	<0.01	<0.01	<0.01	0.12	<0.01	0.34	0.02	0.34	

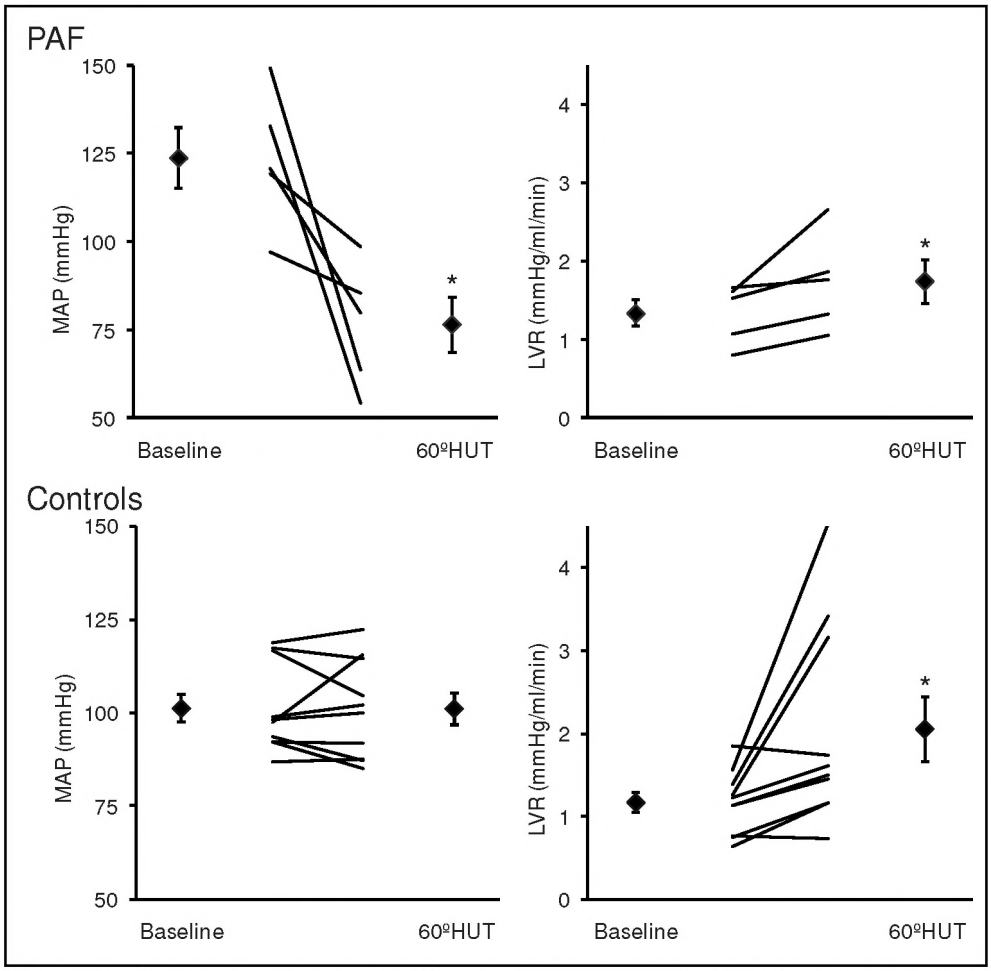
Values represent individual values and mean±SD, respectively. DBH, dopamine-ß-hydroxylase deficiency. PAF, pure autonomic failure. C, controls. MAP, mean arterial blood pressure. LBF, leg blood flow. LVR, leg vascular resistance. SFA, superficial femoral artery diameter. HUT, head-up tilt. L-DOPS, L-threo-dihydroxyphenylserine. ANOVA, indicates the P-values of the repeated measures ANOVA between PAF and controls. *, *post hoc* significantly different from controls. †, significantly different from baseline.

60° head-up tilt

All subjects completed the 10-minute period of 60° HUT. During 60° HUT, leg vascular resistance significantly increased in PAF (30±22%) and controls (72±69%) (*post hoc* p=0.04 and p=0.01, respectively), whilst the increase in leg vascular resistance was not significantly different between groups (Table 3, Figure 1). Controls showed no change in MAP (Table 3, Figure 1), whilst MAP significantly decreased during 60° HUT in PAF (*post hoc* p=0.02) (Table 3, Figure 2). Heart rate increased significantly during 60° HUT in PAF and controls (Table 3). The increase in heart rate was not significantly different between groups (Table 3). In the DBH deficient patients, off and on L-DOPS treatment, leg vascular resistance increased during 60° HUT (DBH1 184% and 413%; DBH2 123% and 57%, respectively) (Table 3, Figure 2).

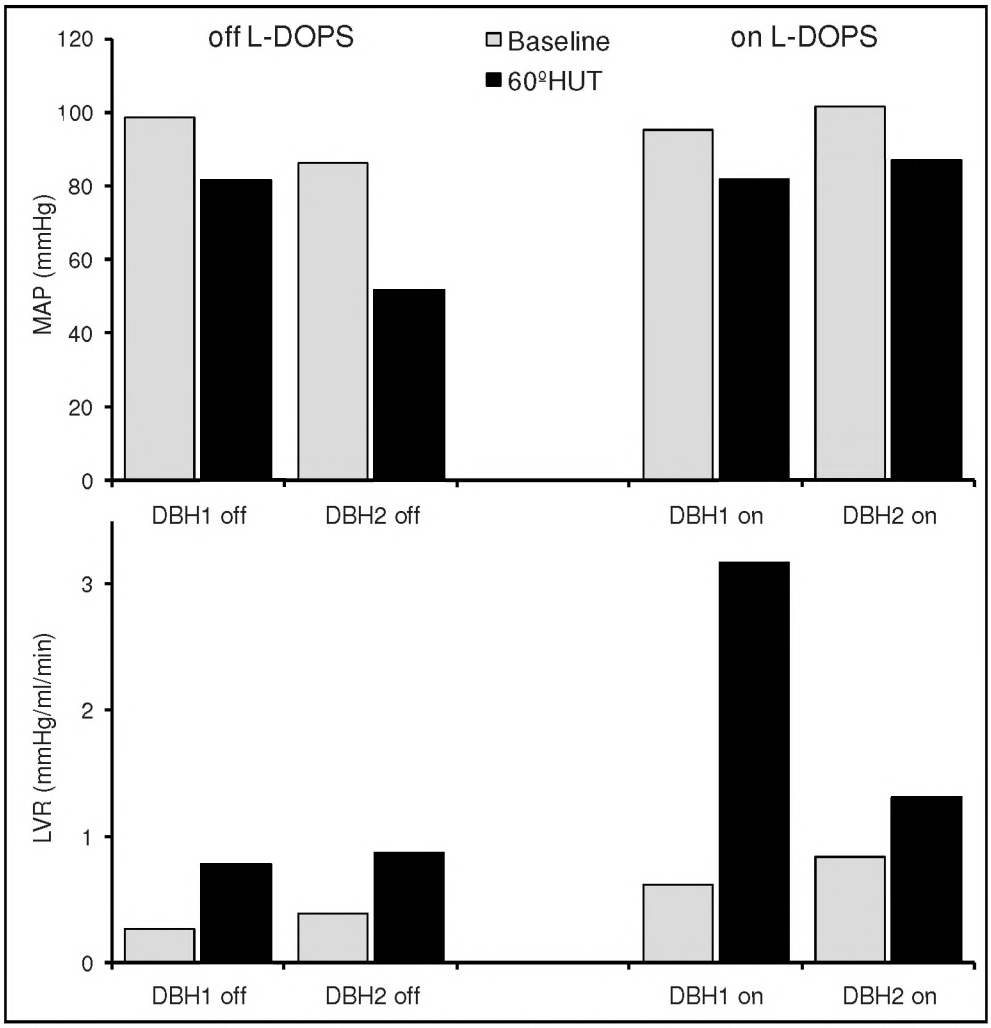
The DBH deficient patients (off and on L-DOPS) showed a decrease in MAP during 60° HUT (Table 3, Figure 2). Heart rate increased in DBH deficient patients off and on L-DOPS (Table 3).

Figure 1. Group and individual mean arterial blood pressure and leg vascular resistance at baseline and during 60° head-up tilt in pure autonomic failure patients and controls.



Diamonds indicate group values (mean±SE) and solid lines indicate individual values. MAP, mean arterial blood pressure. LVR, leg vascular resistance. HUT, head-up tilt. PAF, pure autonomic failure
*, significantly different from baseline.

Figure 2. Mean arterial blood pressure and leg vascular resistance at baseline and during 60° head-up tilt in dopamine-ß-hydroxylase deficient patients, off and on L-DOPS.



DBH, dopamine-ß-hydroxylase deficient patient. MAP, mean arterial blood pressure (MAP). LVR, leg vascular resistance. HUT, head-up tilt.

Stroke volume decreased significantly during 60° HUT in PAF and controls (Table 4). Systemic vascular resistance decreased significantly in PAF but did not change in controls during 60° HUT (Table 4). Controls showed no change in cardiac output, whilst cardiac output decreased during 60° HUT in PAF (*post hoc* p=0.02) (Table 4). The DBH deficient patients showed a decrease in stroke volume and cardiac output

during 60° HUT off and on L-DOPS (Table 4). Systemic vascular resistance decreased during 60° HUT only in DBH2 off L-DOPS (Table 4).

Table 4. Systemic cardiovascular parameters at baseline and during 60° head-up tilt.

Subject	Stroke volume (ml)		Cardiac output (l/min)		SVR (dyn.s/cm^5)	
	Baseline	60° HUT	Baseline	60° HUT	Baseline	60° HUT
DBH1	152	106	7.8	6.1	767	799
with L-DOPS	129	85	6.0	4.7	961	1050
DBH2	93	47	5.2	3.7	1312	1134
with L-DOPS	97	75	6.6	5.2	1249	1343
PAF1	56	46	3.8	3.2	2892	1847
PAF2	53	42	4.2	4.0	2285	1988
PAF3	39	29	2.5	2.0	4416	2175
PAF4	61	48	4.3	3.1	2794	1655
PAF5	68	55	4.7	4.2	2064	1584
PAF (n=5)	**55±13**	**44±11†**	**3.9±0.9**	**3.3±0.9†**	**2890±920***	**1850±242†**
Controls (n=10)	**70±14**	**59±14†**	**4.1±1.1**	**4.2±1.0***	**2158±608**	**2100±597**
	Time	*Interaction*	*Time*	*Interaction*	*Time*	*Interaction*
ANOVA	<0.01	0.85	0.02	<0.01	<0.01	0.01

Values represent individual values and mean±SD, respectively. DBH, dopamine-ß-hydroxylase deficiency. PAF, pure autonomic failure. SVR, systemic vascular resistance. 60° HUT, 60° head-up tilt. L-DOPS, L-threo-dihydroxyphenylserine. ANOVA, indicates the P-values of the repeated measures ANOVA between PAF and controls. *, *post hoc* significantly different from controls. †, significantly different from baseline.

DISCUSSION

To study the importance of the sympathetic nervous system during orthostatic challenges, we examined leg vascular responses during 60° HUT in two distinct groups of chronic postganglionic autonomic disorders. The major findings are 1) an increase in leg vascular resistance during 60° HUT was observed in both postganglionic autonomic failure groups 2) the increase in leg vascular resistance in PAF patients seemed to be lower, although not significantly different from healthy controls, and 3) orthostatic hypotension during 60° HUT was still present in the postganglionic autonomic failure groups, despite the preserved leg vasoconstriction.

These findings indicate that the sympathetic nervous system is not the sole or pivotal mechanism for leg vasoconstriction during orthostatic challenges.

Leg vasoconstrictor mechanisms

To maintain blood pressure during orthostatic challenges, peripheral vasoconstriction is induced by baroreceptor mediated activation of the sympathetic nervous system (1,2), which can be anatomically divided in a preganglionic and postganglionic part (1,3). We demonstrated that leg vasoconstriction in patients with PAF and DBH deficiency is not abolished. In parallel to our findings, a preserved leg vasoconstriction was demonstrated in spinal cord-injured individuals (4-7) with preganglionic autonomic failure due to disruption of the spinal cord. Indeed, the increase in leg vascular resistance during HUT in spinal cord-injured individuals, but also in healthy controls, was unaffected during intra-arterial infusion of phentolamine (α-adrenergic antagonist) (7). The major finding of our study is that an intact sympathetic nervous system is not obligatory for leg vasoconstriction during orthostatic challenges in humans, which reinforces the findings of the previous studies.

As an intact sympathetic nervous system is not obligatory, one may question which mechanism(s) contribute to the leg vasoconstriction in our subjects during 60° HUT. In autonomic disorders with intact sympathetic nerve endings, as in DBH deficiency (10), non-adrenergic neurotransmitters, such as adenosine triphosphate (ATP) and neuropeptide Y (NPY) (16) may contribute to the demonstrated leg vasoconstriction. DBH deficient patients have an intact sympathetic nervous system with a normal increase in baroreflex mediated sympathetic activity during 60° HUT (Table 1) (17). Non-adrenergic neurotransmitters may, therefore, induce vasoconstriction during head-up tilt in DBH deficiency (Table 5). In PAF patients, however, non-adrenergic neurotransmitters are unlikely to contribute to the leg vasoconstriction, given the generalized sympathetic denervation and consequent loss of sympathetic nerve terminals (Tables 1 and 5) (8,9,18). The non-adrenergic neurotransmitters ATP and NPY are co-stored with norepinephrine, simultaneously released from the sympathetic nerve terminal into the synaptic cleft and thought to have a coordinated action with norepinephrine (19). Animal studies have demonstrated the vasoconstrictor capacities of ATP, whereas NPY modulates the action of norepinephrine and ATP (19,20). However, the exact role of noradrenergic neurotransmitters in humans is still unclear.

Also local vasoconstrictor mechanisms may induce vasoconstriction, such as the veno-arteriolar axon reflex (VAR). The VAR is triggered when venous pressure exceeds 25 mmHg, which results in vasoconstriction of the corresponding arteriole, and may importantly contribute to peripheral vasoconstriction during orthostatic challenges (21). The VAR runs through a sympathetic axon and is thought to be α-adrenergic mediated (22). Due to the denervation of the sympathetic postganglionic axons in PAF the VAR cannot contribute to leg vasoconstriction during 60° HUT (Table 1 and 5). In DBH deficiency, based on the absence of norepinephrine, the VAR may only contribute to leg vasoconstriction if it is mediated by non-adrenergic neurotransmitters (Table 1 and 5). This would challenge the general view of the VAR being α-adrenergic mediated (22). However, recent studies suggest that the VAR is not fully α-adrenergic mediated (7,23).

Finally, peripheral vasoconstriction may also be induced via the myogenic response, which is triggered by an increase in transmural pressure across an arteriole (22,24), and is independent of humoral or neuronal influences (25). Therefore, the myogenic response is independent of the sympathetic nervous system. The myogenic response may explain the observed leg vasoconstriction in both PAF and DBH deficiency (Table 5). Similarly, the myogenic response is hypothesized to be responsible for the leg vasoconstriction during HUT and limb dependency in spinal cord-injured individuals (7,26). This redundant local vasoconstrictor mechanism may compensate for the loss of sympathetic mediated vasoconstriction and explain the preserved leg vasoconstriction in patients with autonomic failure.

The results of our study clearly demonstrate that the sympathetic nervous system is important but is not obligatory for leg vasoconstriction during orthostatic challenges. Whilst alternative vasoconstrictor mechanisms may compensate for the loss of sympathetic α-adrenergic vascular control, it is unknown whether and to which extend these mechanisms are present during normal situations or only become active when the sympathetic nervous system fails. Most likely, leg vasoconstriction during orthostatic challenges is caused by a combination of central and local vasoconstrictor mechanisms. When one of these mechanisms fails, the other vasoconstrictor mechanisms can compensate for its loss. The results demonstrate that without control of the sympathetic nervous system, leg vascular resistance can increase by 30% in PAF patients. This 30% increase in leg vascular resistance may be considered as the maximal capacity of non-adrenergic vasoconstrictor mechanisms, most likely the

myogenic response, to contribute to leg vasoconstriction. However, this needs to be studied in detail in future studies.

Table 5. Possible peripheral vasoconstrictor mechanisms in pure autonomic failure and dopamine-ß-hydroxylase deficiency.

Vasoconstrictor mechanisms	PAF	DBH
Baroreflex	−	−
Non-adrenergic neurotransmission*	−	+
Veno-arteriolar axon reflex (VAR)	−	+
Myogenic response	+	+

PAF, pure autonomic failure. DBH, dopamine-ß-hydroxylase deficiency. *, adenosine triphosphate (ATP) and neuropeptide Y (NPY).

Orthostatic hypotension

Despite the leg vasoconstriction during 60° HUT, orthostatic hypotension (≥20 mmHg decrease in systolic or ≥10 mmHg decrease in diastolic pressure within 3 minutes of standing or a 60° HUT (11)) was present in all PAF and DBH deficient patients. Interestingly, the increase in leg vascular resistance during 60° HUT in DBH deficient patients was even larger compared to controls. Orthostatic hypotension in autonomic failure can, therefore, only be partly attributed to changes in the leg vascular response. Potential contributing mechanisms for the orthostatic hypotension in autonomic failure could relate to alterations in the splanchnic vascular bed, an attenuated venoconstriction and/or inadequate plasma volumes. In PAF patients the large decrease in systemic vascular resistance suggests a lack of vasoconstriction in other vascular beds, in contrast to the increase in leg vascular resistance. The vascular resistance of the superior mesenteric artery does not change during head-up tilt in PAF patients, nor does the forearm vascular resistance (27). Suggesting a lack of vasoconstriction of the upper limb and splanchnic vascular bed, although the superior mesenteric artery is only one of three large splanchnic blood vessels. However, systemic vascular resistance decreases, indicating that there should be vasodilation in other vascular beds in PAF patients. Which vascular beds actually demonstrate vasodilation is unknown. Nevertheless, the decrease in systemic vascular resistance during 60° HUT seems to be the major cause of orthostatic hypotension in PAF patients (27-29). Future studies should further examine the exact mechanism explaining the orthostatic hypotension in autonomic failure, which may contribute to the clinical management of these patients.

Baseline values

The baseline leg vascular resistance was not different between PAF and controls, despite generalized sympathetic denervation and low norepinephrine levels in PAF. This could be explained by the use of midodrine (α-adrenoceptor agonists) and to a lesser extend fludrocortisone in PAF, which both increase baseline leg vascular resistance. Similarly, the higher MAP in PAF compared to controls may be explained by increased blood volume by prescribed fludricortisone and sodium. Alternatively, PAF patients may demonstrate physiological adaptations in order to try to prevent orthostatic intolerance (30,31).

Limitations

In our 60° HUT protocol the subjects support their body weight on one leg, allowing the non-weight bearing leg to be relaxed for blood flow measurements. This protocol has been used before (4,32,33), but might increase muscle tensing in the weight-bearing leg (34) and, thereby, increase muscle sympathetic nerve activity (MSNA) by the exercise pressor reflex (35). We have tried to minimize the muscle tensing during 60° HUT by supporting the thigh of the weight-bearing leg by a strap to keep it extended and supporting straps around the waist and chest. Muscle tensing in the weight-bearing leg might result in vasoconstriction in this leg, although it seems to decrease systemic vascular resistance in controls and PAF patients (34). It could, therefore, be that muscle tensing in the weight-bearing leg leads to vasoconstriction and at the same time to systemic vasodilation, explaining our lack of increase in systemic vascular resistance in controls. The demonstrated increase in leg vascular resistance of the non weight-bearing leg could, in that case, be underestimated. Nevertheless, the differences between standing on one or two legs during 60° HUT on central and peripheral vascular responses are unknown.

Our small group sizes resulted in less powerful statistical analyses, especially in DBH deficiency. However, both disorders represent rare autonomic disorders with often serious co-morbidities, while DBH deficient patients rarely cease their medication. Moreover, these unique and carefully selected groups demonstrated comparable cardiovascular responses during 60° HUT. Therefore, increasing our sample size will unlikely change the major outcomes of our study.

Four out of five PAF patients used medication to manage their orthostatic hypotension (Table 2). The use of an α-adrenoceptor agonist (midodrine) increases

basal vascular tone. However, whether this drug altered the vascular tone during orthostatic challenges is unknown. Fludrocortisone and sodium have an effect on circulating volume and fludricortisone has also a lesser effect on vascular tone. It is, therefore, unlikely that the observed leg vasoconstriction during 60° HUT in PAF is a result of medication. Furthermore, one PAF patient did not use any medication but demonstrated similar results as the other PAF patients.

Clinical relevance

The initial cardiovascular responses to orthostatic challenges, i.e. increase in heart rate, cardiac contractility and peripheral vascular resistance, have been allocated to the sympathetic nervous system (2,3,29,36,37). Our results challenge this widely adopted view, since an increase in leg vascular resistance was observed in specific disorders of the postganglionic part of the sympathetic nervous system. Alternative vasoconstrictor mechanisms may compensate for the loss of sympathetic α-adrenergic mediated vasoconstriction in patients with autonomic failure. Furthermore, our results indicate that, despite the increase in leg vascular resistance and optimal pharmacotherapy, orthostatic hypotension was still present in all PAF and DBH deficient patients. This suggests that orthostatic intolerance is unlikely caused by changes in leg vascular resistance.

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General discussion

9

In human evolution, maintaining blood pressure during changes in posture has been an important circulatory achievement. The Autonomic nervous system is thought to play a principal role in the control of blood pressure regulation (1-3). Failure of the autonomic nervous system to control blood pressure regulation may result in an immediate decrease in blood pressure when adopting the upright position, known as orthostatic hypotension (1,3). The baroreceptor mediated increase in sympathetic nervous system activity will result in an increase in heart rate, cardiac contractility and peripheral vascular resistance (1,2). The increase in peripheral vascular resistance in order to maintain blood pressure during the transition from supine to the upright position is scarcely studied. Therefore, we examined the changes in peripheral vascular resistance during the upright posture in individuals with autonomic failure. We also examined the role of peripheral vascular resistance in the episode of hypertension during exaggerated sympathetic activity, i.e. autonomic dysreflexia, in individuals with a spinal cord lesion. In this final chapter, our results are summarized and the consequences of our findings will be discussed and related to other studies.

LEG VASCULAR RESPONSES IN SPINAL CORD INJURY

Basal leg vascular resistance

A spinal cord injury leads to numerous cardiovascular adaptations below the level of the spinal cord lesion (4-6). Supraspinal sympathetic nervous system control of the leg vascular bed is lost in spinal cord injured individuals (SCI) and one might expect a decreased basal leg vascular resistance due to autonomic failure. In contrast, SCI individuals demonstrate an increased basal leg vascular resistance (*Chapters 3-5*) (7-12). We demonstrated in *Chapter 4* a contribution of angiotensin II to the increased basal leg vascular resistance in SCI individuals. Previous studies have demonstrated that the increased basal leg vascular resistance in SCI individuals cannot be explained by adaptations in sympathetic tone (12) or an impaired availability of endothelium-derived NO (7), whereas the vasoconstrictor endothelin-1 contributed significantly (10). Interestingly, endothelin-1 and angiotensin II are closely linked in the regulation of vascular tone. Angiotensin II stimulates endothelin-1 release in cultured endothelial cells (13). The hypertensive effects of angiotensin II in humans might be mediated by stimulation of renal endothelin-1 receptors (14,15). In addition angiotensin II-induced hypertension in rats can be attenuated by endothelin-1

receptor antagonists (16). Therefore, the contribution of angiotensin II and endothelin-1 to the increased basal leg vascular resistance in SCI individuals, could share a common pathway.

We demonstrated in *Chapter 4* that angiotensin II contributed to vascular resistance only in the paralysed and extremely inactive legs and not in the non-paralysed active arms of SCI individuals. Similarly, we demonstrated no contribution of angiotensin II to vascular resistance in healthy active controls. We, therefore, hypothesize that the demonstrated contribution of angiotensin II to the increased basal leg vascular tone in SCI individuals relates to extreme inactivity of the deconditioned legs. Indeed, increased levels of angiotensin II have been demonstrated in bed rest studies (17,18). More importantly, our hypothesis is supported by the consistently demonstrated normalisation of basal leg vascular resistance and the diminished contribution of endothelin-1 to leg vascular resistance after electrical stimulation-assisted exercise in SCI individuals (9,10,19).

The increased basal leg vascular resistance in SCI individuals is not in agreement with the typical finding in autonomic failure. For instance, basal leg vascular resistance in patients with Parkinson's disease (PD) with autonomic failure (*Chapter 7*) and dopamine-ß-hydroxylase deficiency (*Chapter 8*) is lower than in controls (20,21). The lower basal leg vascular resistance in these groups is probably due to a decrease in sympathetic α-adrenergic tone due to sympathetic denervation (PD) (20,22) or the inability to produce noradrenaline (DBH deficiency) (21). This is supported by the fact that the noradrenaline prodrug L-DOPS increased basal leg vascular resistance in DBH deficiency and that the α-adrenoceptor agonist midodrine had a similar effect in pure autonomic failure (PAF) patients (*Chapter 8*). In contrast, the increased basal leg vascular resistance in SCI individuals is not explained by adaptations in sympathetic α-adrenergic tone (12) but by adaptations in the contribution of the humoral vasoconstrictors angiotensin II *(Chapter 4)* and endothelin-1 (10) related to extreme inactivity. The extreme inactivity in SCI individuals and consequent deconditioning of the legs also leads to structural adaptations (vascular atrophy) which may contribute to the increased basal leg vascular resistance. Physical activity level in most individuals with autonomic failure is decreased, however not even close to the extent of the extreme inactivity in SCI individuals. Inactivity, therefore, seems to influence leg vascular tone more than the loss of supraspinal control. More importantly, the effect of inactivity on leg vascular

resistance, i.e. increase in leg vascular resistance, is contrary to the effect of the loss of supraspinal sympathetic control, i.e. decrease in leg vascular resistance.

One might suggest that the increased basal leg vascular resistance in SCI individuals protects them from orthostatic hypotension. In the initial phase after a spinal cord injury ('spinal shock') orthostatic hypotension is present in most SCI individuals and in some SCI individuals the orthostatic hypotension persists over time (23). The increased basal leg vascular resistance in *Chapters 3, 4 and 5* was demonstrated in chronic SCI individuals with low and high spinal cord lesion, but none of these SCI individuals demonstrated orthostatic hypotension. Whether the SCI individuals with orthostatic hypotension have a lower leg vascular resistance is unknown. In line with this, a decrease in leg vascular resistance may increase the susceptibility for orthostatic hypotension in SCI individuals. There are some unpublished cases from our laboratory of SCI individuals who developed orthostatic hypotension after an electrical-stimulation assisted cycling training program aimed to counteract the leg deconditioning. The effects of an electrical-stimulation assisted cycling program on blood pressure responses during orthostatic challenges in SCI individuals are unknown (24).

Autonomic dysreflexia

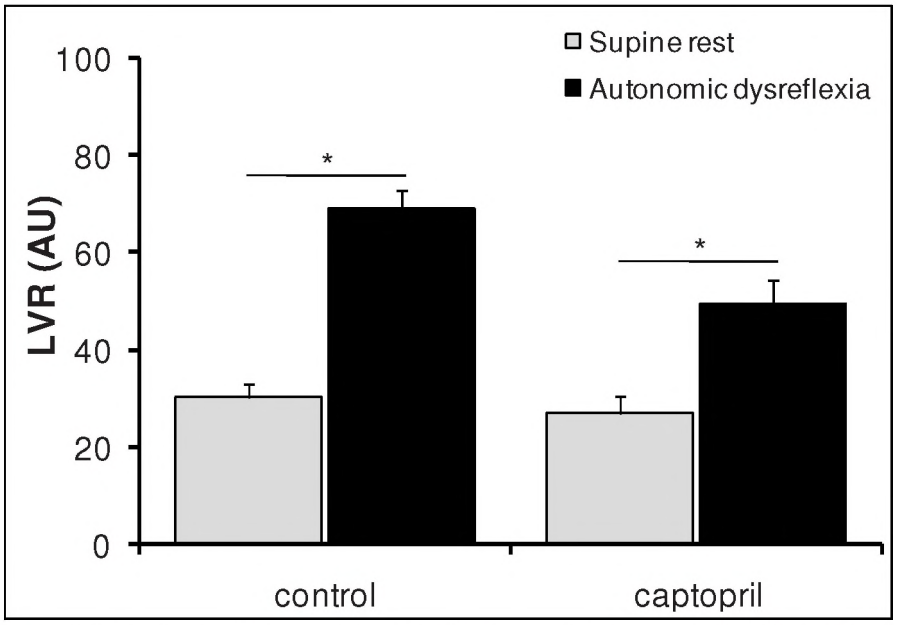
The clinically interesting phenomenon of autonomic dysreflexia can occur in SCI individuals with a spinal cord lesion above the sixth thoracic spinal segment (T6) (4), since a large part of the sympathetic nervous system is without central inhibitory pathways (5,6). Autonomic dysreflexia is characterised by an episode of hypertension induced by exaggerated sympathetic activity caused by a stimulus below the level of the spinal cord lesion (4,6,25,26). Because autonomic dysreflexia is induced by exaggerated sympathetic activity, the widely adopted view is that the vasoconstrictor response is entirely α-adrenergic mediated (5,6,26,27). However, we demonstrated in *Chapter 5* that a residual leg vasoconstriction was present during α-adrenoceptor blockade with intra-arterial phentolamine (α-adrenoceptor antagonist) infusion during autonomic dysreflexia in SCI individuals. We, therefore, believe that autonomic dysreflexia is not entirely α-adrenergic mediated. The rapid and instant increase in blood pressure during autonomic dysreflexia makes other slower vasoconstrictor mechanisms, such as the renin-angiotensin II system (RAS), unlikely. Moreover, plasma levels of renin and angiotensin II did not change during autonomic

dysreflexia (*Chapter 5*), nor does vasopressin (28). Since leg vasoconstriction was still present during α-adrenoceptor blockade, other sympathetic neurotransmitters have to be involved, such as adenosine triphosphate (ATP) and neuropeptide Y (NPY) (29,30). In contrast to SCI individuals, α-adrenoceptor blockade with phentolamine infusion completely blocked the leg vasoconstriction response during exaggerated sympathetic activity evoked by a cold pressor test of the hand in healthy controls (*Chapter 5*). This indicates that supraspinal control of the sympathetic nervous system suppresses the role of non-adrenergic transmission during exaggerated sympathetic activity. Or vice versa, the loss of supraspinal control arouses the role of non-adrenergic transmission to leg vasoconstriction during exaggerated sympathetic activity.

The management of autonomic dysreflexia remains a challenge in clinical practice (25,31). Non-pharmacological strategies, such as removing the visceral, noxious or non-noxious stimulus and a head-up tilt position, remain the first choice followed by pharmacological measures (26,31). In earlier days, α-adrenoceptor antagonists were used (26). Although these drugs are the first choice from a theoretical point of view, serious adverse reactions and the strong vasodilator effect and consequent reduction in blood pressure in the periods in between episodes of autonomic dysreflexia have contributed to the limited use of α-adrenoceptor antagonists. Currently, nifedipine (calcium channel blocker) is used as a primary agent (26,31) and may prevent or control autonomic dysreflexia indicated by a lower blood pressure response (31,32). However, we demonstrated in *Chapter 5* that an α-adrenoceptor antagonist has a more pronounced effect compared to a calcium channel blocker on both the increase in blood pressure and leg vascular resistance. Interestingly, an angiotensin-converting enzyme (ACE) inhibitor (captopril) is also used in the management of autonomic dysreflexia and appears to be safe and effective (33). We demonstrated in *Chapter 4* a contribution of angiotensin II to the increased basal leg vascular resistance in SCI individuals, therefore an ACE inhibitor could also lower leg vascular resistance during autonomic dysreflexia. We, recently, studied the effect of an ACE inhibitor on the leg vasoconstriction during autonomic dysreflexia and demonstrated an attenuated increase in leg vascular resistance (Figure 1). Similar to a calcium channel blocker, the effect of an ACE inhibitor is less pronounced compared to an α-adrenoceptor antagonist. We demonstrated in *Chapter 5* that sympathetic non-adrenergic neurotransmitters contribute to the increase in leg

vascular resistance during autonomic dysreflexia, therefore the development of antagonists of non-adrenergic transmitters might be a target for future management of autonomic dysreflexia. Perhaps these antagonists may appear to be effective in reducing the blood pressure increase during autonomic dysreflexia without significantly lowering the blood pressure in the periods in between episodes of autonomic dysreflexia in SCI individuals. This is a general problem that exists with all available pharmacological agents used nowadays in the management of autonomic dysreflexia.

Figure 1. Leg vascular resistance during autonomic dysreflexia without and with an ACE inhibitor.



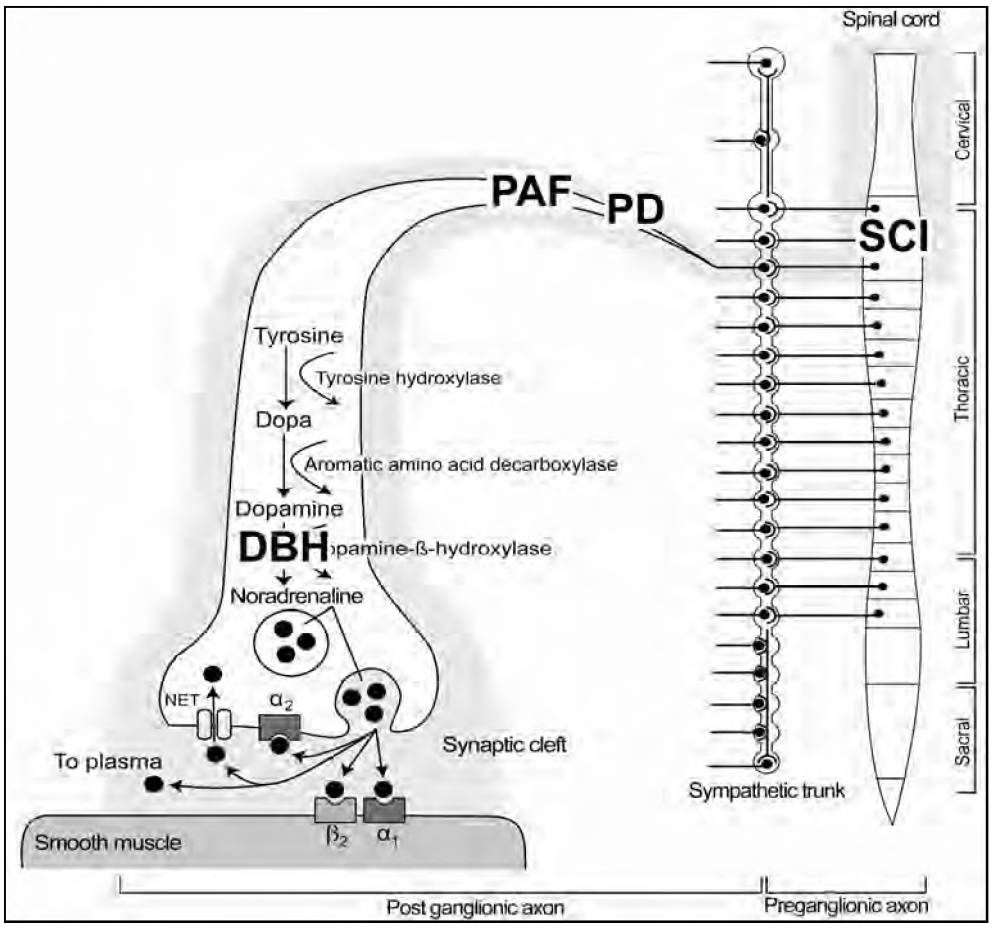
Leg vascular resistance during supine rest and during evoked autonomic dysreflexia in 5 spinal cord-injured individuals under control situation and 90 minutes after an oral dose of captopril (25 mg). AU, arbitrary units. *, significantly difference. *Groothuis, unpublished data.*

LEG VASCULAR RESPONSES IN AUTONOMIC FAILURE

The sympathetic nervous system is thought to play a principal role in the control of blood pressure regulation (1-3) and can be anatomically and functionally divided in a preganglionic and postganglionic (1,34). Autonomic disorders demonstrate selective failure of the different parts of the sympathetic nervous system (Figure 2). The

baroreceptor mediated increase in sympathetic activity, triggered by a drop in blood pressure upon an orthostatic challenge, will result in an increase in heart rate, cardiac contractility and peripheral vascular resistance (1-3). Although the changes in heart rate and cardiac output have been extensively studied during head-up tilt, changes in leg vascular resistance have been scarcely studied. We have, therefore, examined the change in leg vascular resistance during head-up tilt in individuals with autonomic failure due to selective autonomic disorders.

Figure 2. The level of autonomic failure within the sympathetic nervous system of the different autonomic disorders studied in this thesis.



SCI, spinal cord lesion (*Chapters 3-5*). PD, Parkinson's disease (*Chapter 7*). PAF, pure autonomic failure (*Chapter 8*). DBH, dopamine-ß-hydroxylase deficiency (*Chapter 8*).

Non-invasive assessment of leg vascular resistance during head-up tilt

Leg vascular resistance is calculated as the arterial-venous pressure gradient (P_a-P_v) divided by leg blood flow. In the supine position with the leg at or above heart level, the venous pressure is assumed to be close to 0 mmHg. However, during orthostatic challenges a hydrostatic pressure is added to leg arterial as well as to leg venous pressure (3,35,36). The question is how the upright posture affects the arterial-venous pressure gradient. At the onset of an orthostatic challenge, the venous valves will prohibit venous backflow and, thereby, interrupt the formation of a continuous hydrostatic column. However, continued venous filling with blood from the arterioles will, after some time, result in the formation of a continuous hydrostatic column (35-37). Only when continuous hydrostatic columns are formed in both the leg arteries and the leg veins, the assumption can be made that the hydrostatic pressure contributes equally to leg arterial as well as to leg venous pressure (36). The finding in *Chapter 2*, that leg venous pressure during head-up tilt is similar to the calculated hydrostatic pressure after 3-4 minutes of head-up tilt, is of importance to calculate leg vascular resistance with non-invasive measurement techniques during orthostatic challenges. We have, therefore, used these results in the calculation of leg vascular resistance during head-up tilt in this thesis.

Leg vascular responses during head-up tilt

In this thesis we studied the leg vascular responses during head-up tilt in different autonomic disorders with autonomic failure of specific parts of the sympathetic nervous system (Figure 2). In *Chapters 3 and 4* we studied SCI individuals with preganglionic autonomic failure (Table 1). Despite their loss of supraspinal control of the sympathetic nervous system, we demonstrated a preserved increase in leg vascular resistance during head-up tilt. In *Chapter 7* Parkinson's disease (PD) patients with orthostatic hypotension were studied and we demonstrated a preserved increase in leg vascular resistance during head-up tilt. Sympathetic denervation, due to a postganglionic lesion is present in PD with autonomic failure (20,22) (Table 1). Similarly, patients with pure autonomic failure (PAF) have generalised sympathetic denervation due to a postganglionic lesion of the sympathetic nervous system (Table 1), causing widespread autonomic failure (38-40). Despite the loss of sympathetic fibres, we demonstrated in *Chapter 8* a preserved increase in leg vascular resistance in PAF during head–up tilt. In *Chapter 8* we also studied patients with dopamine-ß-

hydroxylase (DBH) deficiency who have a complete absence of noradrenaline with an intact sympathetic nervous system with normal supraspinal control (21,41,42) (Table 1). Surprisingly, we demonstrated in *Chapter 8* a preserved increase in leg vascular resistance during head-up tilt in DBH deficiency despite the absence of noradrenaline. In summary, in autonomic disorders, with autonomic failure due to a lesion in the preganglionic and postganglionic part of the sympathetic nervous system, the increase in leg vascular resistance during head-up tilt is preserved.

Vasoconstrictor mechanisms

The baroreflex mediated increase in sympathetic activity, is thought to be the most important vasoconstrictor mechanism during orthostatic challenges (1-3). However, we have demonstrated in this thesis that individuals with baroreflex failure, due to specific autonomic disorders, have preserved increases in leg vascular resistance during head-up tilt (*Chapters 3, 4, 7 and 8*). This indicates that other vasoconstrictor mechanisms can compensate for the loss of baroreflex mediated leg vasoconstriction. Possible vasoconstrictor mechanisms are non-adrenergic neurotransmission (29,30), humoral factors such as the RAS (3) and local vasoconstrictor mechanisms, such as the veno-arteriolar axon reflex (VAR) (43) and the myogenic response (44,45).

Non-adrenergic neurotransmission

In autonomic disorders with intact sympathetic nerve terminals, such as in SCI individuals (*Chapters 3 and 4*) and DBH deficiency (*Chapter 8*), non-adrenergic neurotransmitters, such as ATP and NPY (29,30), may be involved in the demonstrated increase in leg vascular resistance (Table 1). ATP and NPY are co-stored with noradrenaline, simultaneously released from the sympathetic nerve terminal into the synaptic cleft and thought to have a coordinated action with noradrenaline (30). Animal studies have demonstrated the vasoconstrictor capacities of ATP, whereas NPY modulates the action of noradrenaline and ATP (30,46). However, the exact role of non-adrenergic neurotransmitters in humans is still unclear. Since there is no increase in sympathetic activity during head-up tilt in SCI individuals, indicated by a lack of noradrenaline increase (47,48), it is unlikely that non-adrenergic neurotransmitters are released from the sympathetic nerve terminal. On the contrary, in DBH deficiency there is a normal increase in baroreflex mediated sympathetic activity during head-up tilt (49) and, therefore, non-adrenergic

neurotransmitters may induce leg vasoconstriction in DBH deficiency (Table 1). However, non-adrenergic neurotransmitters should contribute to widespread vasoconstriction but total peripheral resistance decreased during head-up tilt in DBH deficiency (Table 2). It is, therefore, unlikely that non-adrenergic neurotransmission contributes to the demonstrated preserved leg vasoconstriction in DBH deficiency.

Humoral vasoconstrictors

During prolonged orthostatic challenges (>25 min.), the RAS is activated and angiotensin II probably contributes to peripheral vasoconstriction (3,50). However, we demonstrated in *Chapter 4* that angiotensin II does not contribute to the increase in leg vascular resistance during a 10-minute head-up tilt in SCI individuals and controls. Similarly, the vasoconstrictors endothelin-1, vasopressin and aldosterone are unlikely to contribute to the increase in leg vascular resistance during head-up tilt, as they do not increase during short term orthostatic challenges (50,51).

Veno-arteriolar axon reflex

The VAR is a local vasoconstrictor mechanism which may contribute up to 45% of peripheral vasoconstriction during orthostatic challenges in controls (43). The VAR is triggered when venous pressure exceeds 25 mmHg, which results in vasoconstriction of the corresponding arteriole (43). The VAR runs through a sympathetic axon and is thought to be α-adrenergic mediated (44,52). Therefore, the VAR cannot be responsible for the leg vasoconstriction during head-up tilt in autonomic disorders with sympathetic denervation, such as in PD and PAF (*Chapters 7 and 8*) (Table 1). It is unlikely that the VAR plays a role in the increase in leg vascular resistance during head-up tilt in SCI, since the leg vasoconstriction response is still present during α-adrenoceptor blockade (53) (Table 1). Similarly, it is unlikely that the VAR induces leg vasoconstriction in DBH deficiency with a complete absence of noradrenaline (*Chapter 8*). Leg vasoconstriction induced by the VAR, however, could also be due to non-adrenergic neurotransmission, challenging the general view of an α-adrenergic mediated mechanism (52,53). This might be a possibility in SCI individuals and DBH deficiency due to the intact sympathetic nerve endings, although the concerns about non-adrenergic neurotransmitters have been discussed previously.

Table 1. Pathophysiological classification and possible vasoconstrictor mechanisms of the different autonomic disorders

	PAF	PD	DBH	SCI
SNS lesion	Postganglionic	Postganglionic	Postganglionic	Preganglionic
Sympathetic nerves	Degenerative	Degenerative	Intact	Intact
Baseline noradrenaline	Low	Low	Absent	Normal
Noradrenaline release	Low	Low	Absent	Low
Response to noradrenaline	Increased	Increased	Increased	Increased
Baroreflex	–	–	–	–
Non-adrenergic transmission	–	–	+	+
Veno-arteriolar axon reflex	–	–	+*	+*
Myogenic response	+	+	+	+

Spinal cord injury (SCI), Parkinson's disease with autonomic failure (PD), pure autonomic failure (PAF) and dopamine-ß-hydroxylase deficiency (DBH). SNS, sympathetic nervous system. *, via non-adrenergic neurotransmission.

Myogenic response

Another local vasoconstrictor mechanism is the myogenic response, which is triggered by an increase in transmural pressure across an arteriole (44,45). The myogenic response is independent of humoral and neuronal influences (54), and, thus, independent of the sympathetic nervous system. In SCI individuals the myogenic response is responsible for the increase in leg vascular resistance during limb dependency (55). The autonomic disorders studied in this thesis, all have an unimpaired myogenic response (Table 1) and, therefore, the myogenic response may be responsible for the demonstrated preserved increase in leg vascular resistance during head-up tilt in autonomic failure where baroreflex control is absent.

Orthostatic hypotension

Blood pressure is defined as the product of cardiac output (heart rate times stroke volume) and total peripheral resistance. Although the increase in peripheral resistance is important to maintain blood pressure during orthostatic challenges (2,56), the preserved increase in leg vascular resistance could not prevent orthostatic hypotension in PD, PAF and DBH deficiency (*Chapters 7 and 8*) (Table 2). There are some possible explanations for our findings.

Table 2. Changes in cardiovascular parameters during head-up tilt.

	ΔMAP (mmHg)	ΔHR (bpm)	ΔSV (ml)	ΔCO (l/min)	ΔTPR (dyn.s/cm^5)	ΔLVR (mmHg/min/ml)
30° head-up tilt						
Controls	2±5	3±4	-8±6	-0.2±0.4	101±168	0.71±0.81
SCI	6±10	4±3	-5±6	0.0±0.4	69±176	0.63±0.50
60° head-up tilt						
PD-OH	4±9	9±4	-6±7	0.0±0.6	27±648	0.88±0.56
PD+OH	-14±8	9±4	-19±5	-0.7±0.2	-9.4±133	0.34±0.37
DBH	-26±12	14±11	-46±0	-1.6±0.1	-89±126	0.49±0.01
PAF	-48±34	5±8	-11±2	-0.6±0.4	-1040±879	0.40±0.38

SCI, spinal cord-injured individuals. PD-OH, Parkinson's disease without orthostatic hypotension. PD+OH, Parkinson's disease with orthostatic hypotension. DBH, dopamine-ß-hydroxylase deficiency PAF, pure autonomic failure. MAP, mean arterial blood pressure. HR, heart rate. SV, stroke volume. CO, cardiac output. TPR, total peripheral resistance. LVR, leg vascular resistance.

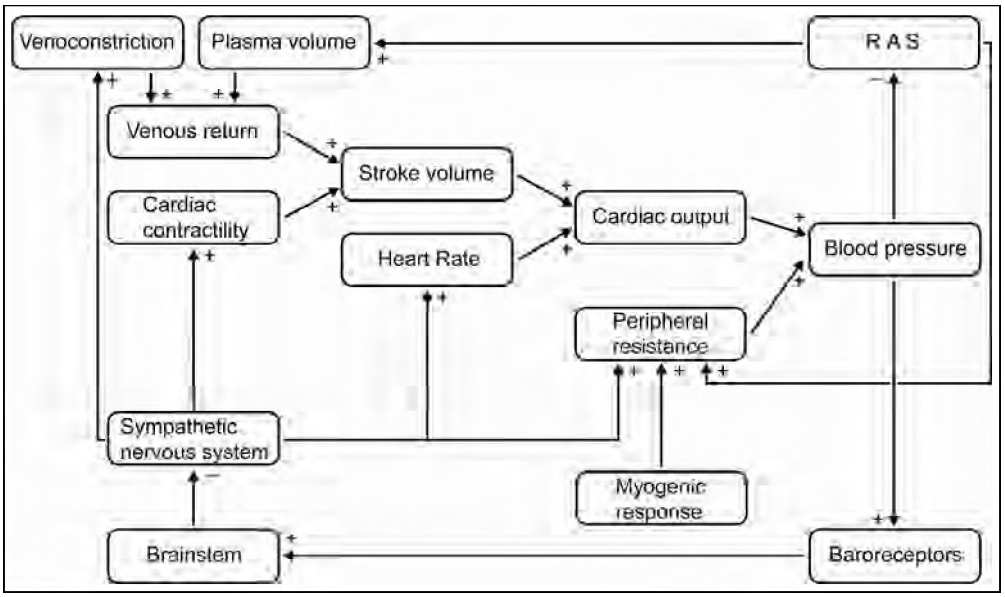
The increase in leg vascular resistance during head-up tilt was attenuated in PD with orthostatic hypotension, PAF and DBH deficiency (Table 2). This attenuated leg vasoconstriction could contribute to orthostatic hypotension. More importantly, we determined leg vascular resistance and not total peripheral vascular resistance. Besides the leg vascular bed, the splanchnic vascular bed importantly contributes to total peripheral vascular resistance. The vascular responses of the splanchnic vascular bed in autonomic failure are unknown. However, we found small to large decreases in total peripheral vascular resistance during head-up tilt (Table 2). Especially in PAF, the decrease in total peripheral vascular resistance was large and might contribute to their orthostatic hypotension. Since leg vascular resistance increased in these groups, the decrease in total peripheral resistance must be due to vasodilation in other vascular beds. It is unknown in which vascular beds vasodilation occurs during orthostatic challenges in autonomic failure, but it might be that the splanchnic vascular bed plays an important role.

Basal leg vascular resistance was lower in PD with orthostatic hypotension and DBH deficiency compared to healthy controls (*Chapters 7 and 8*). Therefore, despite the preserved leg vasoconstriction, the leg vascular resistance during head-up tilt was still lower in PD with orthostatic hypotension and DBH deficiency compared to healthy controls. As a result of the lower leg vascular resistance, vascular capacity is increased and will result in a relative low plasma volume (57). Despite the increased plasma volume in PD with orthostatic hypotension (*Chapter 7*), it might still be a

relative low plasma volume. The relative low plasma volume could contribute to the lower stroke volume in PD with orthostatic hypotension and DBH deficiency (Table 2) and, consequently, contribute to orthostatic hypotension.

Blood pressure is defined as the product of cardiac output and total peripheral resistance. Therefore, blood pressure is not only depending on peripheral resistance but also on cardiac output. A decrease in cardiac output may, therefore, contribute to orthostatic hypotension in autonomic failure. Cardiac output is defined as heart rate times stroke volume and especially stroke volume seems to be essential to maintain cardiac output. Heart rate increased in all groups during head-up tilt, but still cardiac output decreased in PD with orthostatic hypotension, DBH deficiency and PAF (Table 2). Stroke volume decreased in PD with orthostatic hypotension, PAF and DBH deficiency (Table 2) and may contribute to orthostatic hypotension. Since stroke volume depends on the venous return of blood to the heart, a larger decrease in venous return might contribute to orthostatic hypotension in autonomic failure. An absent increase in cardiac contractility, due to sympathetic denervation of the heart in autonomic failure, may also contribute to a lower stroke volume during (Figure 2).

Figure 2. Schematic representation of blood pressure control.

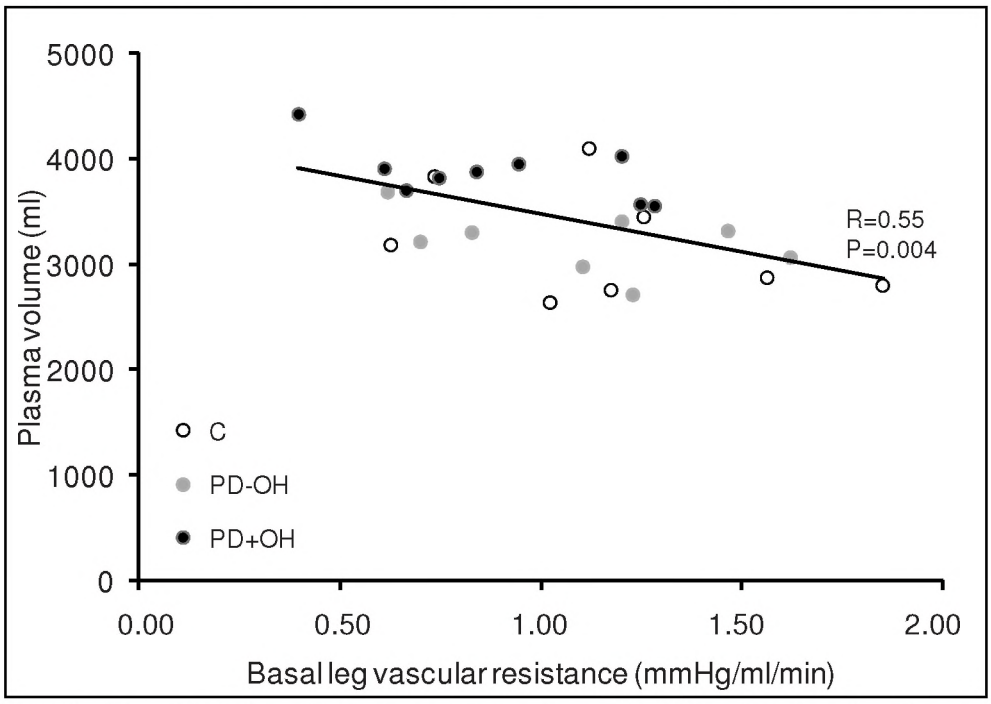


In summary, the orthostatic hypotension in autonomic failure may be explained by an attenuated increase in leg vascular resistance, vasodilation in other vascular beds, a low basal leg vascular resistance, an absent increase in cardiac contractility, a decreased venous return or a combination of these options. Interestingly, it may well be that the contributions of the aforementioned possibilities are different for each autonomic disorder.

Contribution of the venous system

In this thesis we have focused on the arterial leg vasoconstriction during head-up tilt. However, as mentioned in the previous paragraph, the venous system might play a principal role in orthostatic hypotension in autonomic failure. During orthostatic challenges blood is translocated to the dependent venous vasculature (3,35), lowering stroke volume and consequently cardiac output (figure 2). The mechanism of a decrease in venous return in autonomic failure may have different causes. First, baroreflex mediated sympathetic activity does not only result in arterial vasoconstriction but also in venoconstriction (Figure 2), which is mainly directed to the splanchnic vascular bed (3,58). Baroreflex failure will probably result in an absent venoconstriction of the splanchnic vascular bed, thereby reducing the venous return (3). Secondly, an increase in venous pooling can reduce venous return, however in *Chapter 7* we demonstrated no increase in venous pooling in the lower legs of PD patients with orthostatic hypotension. Finally, an inadequate plasma volume may contribute to a reduction in venous return (1,57) (Figure 2). Autonomic failure might influence plasma volume since the sympathetic nervous system plays an important role in volume homeostasis via direct renal innervation and via an increase in RAS activity (59). In *Chapter 7*, however, we demonstrated a higher plasma volume in PD patients with orthostatic hypotension, similarly to one previous study in autonomic failure (60). The increased plasma volume in PD patients with orthostatic hypotension might be a compensatory mechanism for the lower basal leg vascular resistance, which is supported by a strong correlation (Figure 3). Nonetheless, the PD patients were unable to maintain blood pressure during an orthostatic challenge. In summary, although the exact mechanism of orthostatic hypotension in autonomic failure is unknown, we have demonstrated that adaptations in leg vascular resistance are not the main cause.

Figure 3. Correlation between basal leg vascular resistance and plasma volume.



Controls (C; n=8), Parkinson's disease patients without (PD-OH; n=9) and with orthostatic hypotension (PD+OH; n=9). The P value refers to the Pearson's correlation coefficient (R) calculated for all subjects (n=26).

IS THE SYMPATHETIC NERVOUS SYSTEM OBLIGATORY FOR STANDING?

The results described in this thesis demonstrate that in autonomic disorders with selective failure of different levels of the autonomic nervous system (Figure 1), an increase in leg vascular resistance is still present during orthostatic challenges despite sympathetic nervous system failure. This indicates that alternative vasoconstrictor mechanisms, most likely the myogenic response, can compensate for the loss of baroreflex mediates sympathetic vasoconstriction. Even in acute autonomic failure, simulated by α-adrenoceptor blockade in healthy controls, leg vasoconstriction was preserved during head-up tilt (53). This suggests that alternative vasoconstrictor mechanisms are present in the legs. It seems that the supraspinal control of the sympathetic mediated α-adrenergic leg vasoconstriction overrules the alternative local vasoconstrictor mechanisms. This is illustrated by the attenuated increase in leg vascular resistance in situations with an increased sympathetic nervous system

activity, such as in aging (*Chapter 6*). Furthermore, during exaggerated sympathetic activity with supraspinal control the vasoconstrictor response was completely abolished with α-adrenoceptor blockade, in contrast to sympathetic activity without supraspinal control (*Chapter 5*).

However, in autonomic failure the increase in leg vascular resistance is inadequate to maintain blood pressure in the upright posture. This suggests that the baroreflex mediated increase in sympathetic activity resulting in vasoconstriction in vascular beds, other than the legs, cardiac contractility and venoconstriction is obligatory to maintain blood pressure in the upright posture. It might be that the evolutionary process of maintaining blood pressure in the upright position in humans is not yet completely accomplished. Most systems in the human body do not depend on one physiological mechanism. Maintaining blood pressure in the upright position in humans seems to rely mainly on the sympathetic nervous system, although other mechanisms try to compensate for the loss of sympathetic nervous system control of blood pressure regulation.

IMPLICATIONS OF THE STUDIES AND FUTURE DIRECTIONS

Secondary complications of a spinal cord injury are of significance for the quality of life of a SCI individual. Understanding the pathophysiological mechanisms of these secondary complications is, therefore, essential for the clinical management of SCI individuals. Pressure sores and poor wound healing is a major problem in SCI individuals. The increased basal leg vascular resistance in SCI individuals may importantly contribute to the development of these secondary complications. In combination with previous research of our department we have now indentified two major vasoconstrictor pathways which contribute to the increased basal leg vascular resistance. These two vasoconstrictors, angiotensin II and endothelin-1, are closely connected in the regulation of vascular tone. We hypothesise that lowering the basal leg vascular resistance with receptor antagonists of angiotensin II or endothelin-1 may lower or prevent the development of pressure sores and improve wound healing. Since the contribution of these vasoconstrictors seems to be related to extreme physical inactivity, one might also try to improve physical activity by, for instance, electrical stimulation-assisted exercise. To prove this hypothesis we first have to assess leg vascular resistance in SCI individuals with pressure sores. More ideally, we should

assess whether the SCI individuals with high basal leg vascular resistance have an increased risk in developing pressure sores. One step further is to assess whether angiotensin II and endothelin-1 antagonists or physical activity can improve or accelerate wound and pressure sores healing. The final step would be to try to prevent the development of pressure sores by treating the SCI individuals at risk. We should make one important remark, angiotensin II and endothlein-1 receptor antagonists can induce hypotension and, therefore, blood pressure monitoring is of utmost importance.

Autonomic dysreflexia is another serious secondary complication of a spinal cord injury. Prevention of the development of autonomic dysreflexia by informing the patient, patient carers and medical personnel is the primary action in autonomic dysreflexia management. The first step during autonomic dysreflexia is removing the triggering factor and start non-pharmacological strategies. Only when these strategies fail to lower blood pressure, pharmacological measures are indicated. However, the management of autonomic dysreflexia remains a challenge. Our results suggest a new target for future autonomic dysreflexia management, i.e. non-adrenergic neurotransmitters.

Patients with autonomic failure often complain of orthostatic hypotension being the most disabling symptom. Treatment of orthostatic hypotension in autonomic failure is a challenge and hardly evidence based (1,61). Our results suggest that an attenuated increase in leg vascular resistance does not contribute to orthostatic hypotension in autonomic failure. However, in some autonomic disorders basal leg vascular resistance is low and, despite leg vasoconstriction, remains lower during orthostatic challenges as compared to controls. It might be that the low basal leg vascular resistance contributes to the orthostatic hypotension. Increasing the basal leg vascular resistance could, therefore, be an effective strategy, although the results in PAF patients, with α-adrenergic agonist treatment, suggest otherwise. We demonstrated that a compensatory vasoconstrictor mechanism, the myogenic response, might be responsible for the observed increase in leg vascular resistance in autonomic failure. Future research should focus on trying to improve the vasoconstrictor capacities of this compensatory mechanism in order to try to manage orthostatic hypotension. Non-pharmacological interventions, such as physical activity, or pharmacological interventions in order to improve the vasoconstrictor capacities of the myogenic response could be studied. As discussed, a decrease in

cardiac output, probably due to a decrease in venous return, is an important additional factor in orthostatic hypotension in individuals with autonomic failure. The mechanism of the decreased cardiac output in autonomic failure is unclear and, therefore, of interest for future studies. As a first step, interventions that increase venous return should be studied for their efficacy to treat orthostatic hypotension in autonomic failure.

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Summary
Nederlandse samenvatting

10

SUMMARY

The upright posture of humans greatly challenges the control of blood pressure regulation. The autonomic nervous system plays a principal role in the control of blood pressure regulation via changes in sympathetic nervous system activity. Baroreflex mediated increases in sympathetic nervous system activity leads to changes in heart rate, cardiac contractility and peripheral vascular resistance. Surprisingly, the changes in peripheral vascular resistance are scarcely studied. Therefore, the general aim of this thesis was to examine the role of the autonomic nervous system in a specific part of the blood pressure regulation, i.e. peripheral vascular control, in individuals with autonomic failure, with a special interest in individuals with a spinal cord lesion.

Throughout this thesis we have calculated leg vascular resistance, as measure for peripheral vascular control, during head-up tilt as the arterial-venous pressure gradient divided by blood flow. In *Chapter 2* we have examined the changes in intravenous pressure during head-up tilt in healthy controls. Intravenous pressure measured in the great saphenous vein was similar to the calculate hydrostatic pressure during 30° and 70° head-up tilt. The hydrostatic pressure contributes, therefore, equally to leg arterial as well as to leg venous pressure during head-up tilt.

In *Chapter 3* we examined the changes in leg vascular resistance during 30° head-up tilt in spinal cord-injured (SCI) individuals. Despite their central autonomic failure, due to the disruption of the spinal cord, SCI individuals had a preserved leg vasoconstriction during 30° head-up tilt. This finding demonstrates that humans are able to increase their leg vascular resistance independently of supraspinal sympathetic control.

Although SCI individuals have central autonomic failure they demonstrate an increased basal leg vascular resistance. In *Chapter 4*, we demonstrated a contribution of angiotensin II to the increased basal leg vascular resistance in SCI individuals. Angiotensin II did not contribute to forearm vascular resistance in SCI individuals nor to leg and forearm vascular resistance in healthy controls. The contribution of angiotensin II to the increased leg vascular resistance in SCI individuals might, therefore, relate to the extreme inactivity of the paralyzed legs. Furthermore, we

demonstrated in *Chapter 4* that angiotensin II does not contribute to the preserved leg vasoconstriction in SCI individuals during 30° head-up tilt. Nor does it contribute to leg vasoconstriction during 30° head-up tilt in healthy controls.

Because SCI individuals have an intact sympathetic nervous system with no supraspinal control, the interesting clinical phenomenon of autonomic dysreflexia can occur. This episode of hypertension in SCI individuals was thought to be α-adrenergic mediated. We demonstrated in *Chapter 5* that the increase in leg vascular resistance during autonomic dysreflexia in SCI individuals is not entirely α-adrenergic mediated. This finding demonstrates that the leg vasoconstriction during autonomic dysreflexia in SCI individuals is partly explained by non-adrenergic neurotransmitters, such as adenosine triphosphate (ATP) and neuropeptide Y. The increase in leg vascular resistance during a cold pressure test of the hand in healthy controls was completely α-adrenergic mediated, suggesting that non-adrenergic neurotransmission is suppressed by supraspinal control.

Older men have an augmented increase in total peripheral resistance during head-up tilt despite their chronically elevated sympathetic nervous system activity. However, in *Chapter 6* we demonstrated an attenuated increase in leg vascular resistance during 30° head-up tilt in older men compared to young controls. The attenuated increase in leg vascular resistance may be part of the explanation of orthostatic hypotension with advancing age.

In *Chapter 7* we examined the changes in leg vascular resistance during 60° head-up tilt and measured plasma volume in Parkinson's disease patients with and without orthostatic hypotension. Parkinson's disease patients with orthostatic hypotension demonstrated a lower basal leg vascular resistance in combination with a larger plasma volume compared to Parkinson's disease patients without orthostatic hypotension and healthy controls. Parkinson's disease patients with orthostatic hypotension demonstrated a preserved leg vasoconstriction during 60° head-up tilt, which was not different from Parkinson's disease patients without orthostatic hypotension and healthy controls. These findings demonstrate that orthostatic hypotension in Parkinson's disease is not due to an attenuated leg vasoconstriction or an inadequate plasma volume, but may partly be explained by a lower basal leg vascular resistance.

In *Chapter 8* we examined the changes in leg vascular resistance during 60° head-up tilt in two selective autonomic disorders with autonomic failure of the postganglionic (pure autonomic failure) and neurotransmitter (dopamine-ß-hydroxylase deficiency) part of the sympathetic nervous system. Despite their autonomic failure, we demonstrated a preserved leg vasoconstriction during 60° head-up tilt in pure autonomic failure and dopamine ß-hydroxylase deficient patients. This finding indicate that the sympathetic nervous system is not obligatory for leg vasoconstriction during orthostatic challenges.

NEDERLANDSE SAMENVATTING

Eén van de belangrijkste momenten in de evolutie van de mens is het gaan staan en lopen op twee benen. De rechtopstaande houding geeft de mens een groot voordeel, maar tegelijkertijd is het een uitdaging voor de regelsystemen die noodzakelijk zijn om de bloeddruk te kunnen handhaven. Het autonoom zenuwstelsel heeft in dit proces een belangrijke rol.

Wanneer wij rechtop gaan staan zal onder invloed van de zwaartekracht meer bloed de bloedvaten onder het niveau van het hart instromen. Hierdoor wordt het hart minder gevuld met bloed en kan er minder bloed vanuit het hart de grote lichaamsslagader (aorta) worden gepompt. Dit leidt tot een bloeddrukdaling die opgemerkt zal worden door bloeddruksensoren in de halsslagaders en de aorta. Deze sensoren verhogen de activiteit van het sympathische zenuwstelsel, een onderdeel van het autonoom zenuwstelsel. Deze verhoogde activiteit leidt tot een toename van de pompkracht van het hart, een toename van de hartslag en een toename van de perifere vaatweerstand. Binnen enkele hartslagen zal hierdoor de bloeddrukdaling worden tegengegaan en de bloeddruk gehandhaafd blijven.

Wanneer het bloeddrukregelsysteem faalt kan dit leiden tot een bloeddrukdaling tijdens het rechtop gaan staan, wat orthostatische hypotensie wordt genoemd. Dit proefschrift beschrijft het onderzoek naar de rol van veranderingen in de perifere vaatweerstand in het handhaven van de bloeddruk bij personen met een aandoening van het sympathisch zenuwstelsel (autonoom falen), met speciale aandacht voor personen met een dwarslaesie.

Als maat voor de perifere vaatweerstand, maken we in dit proefschrift gebruik van de vaatweerstand in de benen. De vaatweerstand in de benen kan tijdens zogenaamde kantelproef berekend worden door het verschil in druk tussen slagader en ader te delen door de bloedstroom. In *Hoofdstuk 2* hebben we de veranderingen in intraveneuze druk gemeten tijdens een kantelproef bij gezonde personen. Hieruit kwam naar voren dat bij kantelhoeken van 30° en 70° de intraveneuze druk gelijk was aan de berekende hydrostatische druk. Tijdens een kantelproef heeft de hydrostatisch druk dus eenzelfde invloed op de druk in de slagader als op de druk in de ader van het been.

In *Hoofdstuk 3* hebben we de veranderingen in de vaatweerstand in de benen bij personen met een dwarslaesie gemeten. Deze personen hebben door schade aan het ruggenmerg geen centrale controle over het sympathische zenuwstelsel onder het niveau van de dwarslaesie. Ondanks verlies van controle over het sympathisch zenuwstelsel vinden wij tijdens een kantelproef een toename in de vaatweerstand in de benen bij personen met een dwarslaesie. Dit resultaat toont aan dat de mens de vaatweerstand in de benen kan verhogen, onafhankelijk van centrale controle over het sympathisch zenuwstelsel.

Personen met een dwarslaesie hebben in rust een hogere vaatweerstand in de benen in vergelijking met gezonde controles. In *Hoofdstuk 4* laten we zien dat angiotensine II, een vaatweerstand verhogende stof, een rol speelt in deze verhoogde vaatweerstand bij personen met een dwarslaesie. Angiotensine II speelt echter geen rol in de vaatweerstand in de armen van personen met een dwarslaesie en ook niet in de vaatweerstand in de armen en benen van gezonde personen. De verhoogde bijdrage van angiotensine II aan de vaatweerstand in de benen van personen met een dwarslaesie kan daarom gerelateerd zijn aan de extreme inactiviteit van de verlamde benen. Tevens laten we in *Hoofdstuk 4* zien dat angiotensine II geen rol speelt in de toename van de vaatweerstand in de benen tijdens een kantelproef bij zowel personen met een dwarslaesie als gezonde personen.

Personen met een dwarslaesie hebben onbeschadigde sympathische zenuwvezels onder het niveau van de dwarslaesie. Overactiviteit van deze zenuwvezels kan zorgen voor het optreden van het interessante klinische fenomeen van autonome dysreflexie. Dit betreft een episode van een verhoogde bloeddruk in personen met een dwarslaesie, veroorzaakt door een pijnprikkel onder het niveau van de dwarslaesie, zoals bijvoorbeeld een overvolle blaas of een blaasontsteking. Gedacht wordt dat een overactiviteit van het sympathische zenuwstelsel onder het niveau van de dwarslaesie leidt tot een forse toename in vaatweerstand en daarmee een stijging van de bloeddruk. Aangenomen wordt dat de toename in vaatweerstand ontstaat via het vrijkomen van noradrenaline uit de uiteinden van de sympathische zenuwvezels. In *Hoofdstuk 5* tonen we aan dat de toename van de vaatweerstand in de benen tijdens autonome dysreflexie bij personen met een dwarslaesie niet alleen door het vrijkomen van noradrenaline veroorzaakt wordt. Ook de non-adrenerge neurotransmitters adenosine trifosfaat (ATP) en neuropeptide Y, stoffen die ook vrijkomen uit de uiteinden van sympathische zenuwvezels, kunnen voor een deel de toename van de

vaatweerstand in de benen tijdens autonome dysreflexie verklaren. Bij gezonde personen was de toename van de vaatweerstand tijdens een verhoging van de activiteit van het sympathisch zenuwstelsel door middel van een pijnprikkel wel geheel te verklaren door het vrijkomen van noradrenaline. Deze resultaten suggereren dat centrale controle over het sympathisch zenuwstelsel het vrijkomen van non-adrenerge neurotransmitters onderdrukt.

Ondanks een chronisch verhoogde activiteit van het sympathisch zenuwstelsel, hebben ouderen een verhoogde toename van de totale perifere vaatweerstand tijdens een kantelproef. In *Hoofdstuk 6* laten wij echter zien dat tijdens deze kantelproeven gezonde oudere mannen een verminderde toename van de vaatweerstand in de benen hebben in vergelijking met gezonde jonge mannen. De verminderde toename van de vaatweerstand in de benen kan een verklaring zijn voor het frequenter optreden van orthostatische hypotensie bij het ouder worden.

In *Hoofdstuk 7* hebben we naast de veranderingen in de vaatweerstand in de benen tijdens een kantelproef bij personen met de ziekte van Parkinson met en zonder orthostatische hypotensie ook het plasma volume gemeten. In vergelijking met personen met de ziekte van Parkinson zonder orthostatische hypotensie en gezonde personen hebben personen met de ziekte van Parkinson met orthostatische hypotensie in rust een lagere vaatweerstand in de benen gecombineerd met een hoger plasma volume. Tijdens een kantelproef is er bij personen met de ziekte van Parkinson met orthostatische hypotensie een toename in de vaatweerstand in de benen. Echter deze toename is niet verschillend van personen met de ziekte van Parkinson zonder orthostatische hypotensie en gezonde personen. Deze resultaten laten zien dat orthostatische hypotensie in personen met de ziekte van Parkinson niet veroorzaakt wordt door een verminderde toename van de vaatweerstand in de benen of door een inadequaat plasma volume, maar deels te verklaren is door een verlaagde vaatweerstand in de benen in rust.

In *Hoofdstuk 8* hebben we de veranderingen in de vaatweerstand in de benen tijdens een kantelproef gemeten bij personen met twee selectieve aandoeningen van het sympathisch zenuwstelsel. Deze personen hebben autonoom falen door een aandoening van de post-ganglionaire sympathische zenuwvezels (puur autonoom

falen) en door een afwezigheid van noradrenaline (dopamine-ß-hydroxylase deficiëntie). Ondanks het falen van het sympathisch zenuwstelsel, vinden we bij deze personen een toename van de vaatweerstand in de benen tijdens een kantelproef. Deze resultaten wijzen erop dat het sympathische zenuwstelsel niet noodzakelijk is voor een toename van de vaatweerstand in de benen tijdens rechtop gaan staan.

Dit proefschrift laat zien dat ondanks autonoom falen door verschillende aandoeningen van het sympathisch zenuwstelsel de mens tijdens een kantelproef een toename heeft van de vaatweerstand in de benen. Deze toename van de vaatweerstand wordt waarschijnlijk veroorzaakt door lokale regelsystemen in de bloedvaten van de benen en is onafhankelijk van het autonoom zenuwstelsel. Deze lokale regelsystemen proberen de bloeddruk te handhaven tijdens rechtop gaan staan wanneer het sympathisch zenuwstelsel faalt. Echter de toename van de vaatweerstand in de benen bij mensen met autonoom falen is onvoldoende om de bloeddruk te handhaven.

Curriculum Vitae

CURRICULUM VITAE

Jan Groothuis, de auteur van dit proefschrift, werd op 10 augustus 1977 geboren te Rheden. Hij behaalde in 1996 zijn VWO diploma aan het Christelijk Streeklyceum in Ede. Aansluitend ging hij Biomedische Gezondheidswetenschappen studeren aan de Radboud Universiteit Nijmegen, waar hij zich specialiseerde in de Bewegingswetenschappen en Fysiologie. In het laatste jaar van zijn studie deed hij onderzoek naar de cardiovasculaire reacties tijdens een kantelproef bij personen met een dwarslaesie onder leiding van professor M Hopman (afdeling fysiologie, UMC St Radboud te Nijmegen). Na zijn afstuderen in 2000, stroomde hij door in het verkorte doctoraal programma van de studie Geneeskunde aan de Radboud Universiteit Nijmegen. In 2004 behaalde hij zijn arts-examen, waarna hij eerst als ANIOS neurologie in de Isala Klinieken te Zwolle heeft gewerkt. In september 2005 begon hij in de St Maartenskliniek te Nijmegen aan zijn opleiding tot revalidatiearts (opleiders: drs. D van Kuppevelt en professor A Geurts) gecombineerd met zijn promotieonderzoek '*Vascular control in individuals with autonomic failure*' in het UMC St Radboud middels een AGIKO stipendium van ZonMw. Zijn promotieonderzoek werd begeleid door professor M Hopman, professor P Smits en dr. G Rongen, een samenwerking van de afdelingen Fysiologie en Farmacologie-Toxicologie. Voor zijn onderzoek ontving hij reisbeurzen van de American Autonomic Society, European Federation of Autonomic Societies en de Werkgroep voor Syncope en Autonome aandoeningen. In 2010 mocht hij de 'Streeten Travel Fellowship Award' van de American Autonomic Society in ontvangst nemen. Op dit moment is hij bezig met het afronden van zijn opleiding tot revalidatiearts en betrokken bij verschillende onderzoeksprojecten van de afdeling Fysiologie van het UMC St Radboud.

CURRICULUM VITAE

Jan Groothuis, the author of this thesis, was born on August 10, 1977 in Rheden, Netherlands. In 1996 he obtained his '*VWO*' diploma at the '*Christelijk Streeklyceum*' in Ede. Afterwards he studied Biomedical Health Sciences at Radboud University Nijmegen. In his last year, he studied the cardiovascular responses during head-up tilt in spinal cord-injured individuals under supervision of Professor M Hopman (Department of Physiology, Radboud University Nijmegen Medical Centre). After obtaining his Master degree in 2000, he studied Medicine at Radboud University Nijmegen. In 2004 he obtained his Medical Degree and started working as a resident in the Neurology department of the '*Isala Klinieken*' in Zwolle. In September 2005 he commenced his residency in Rehabilitation at '*St Maartenskliniek*' in Nijmegen (supervisors: D. van Kuppevelt and Professor A Geurts) combined with his PhD research project 'Vascular control in individuals with autonomic failure' at the Physiology department at Radboud University Nijmegen Medical Centre, supported by an '*AGIKO*' stipendium by '*ZonMw*'. His PhD research project was supervised by Professor M Hopman, Professor P Smits and dr. G Rongen, a collaboration of the departments of Physiology and Pharmacology-Toxicology. He was awarded for his PhD research with travel grants from the American Autonomic Society, European Federation of Autonomic Societies and 'Werkgroep voor Syncope and Autonome aandoeningen'. In 2010 he received the 'Streeten Travel Fellowship Award' from the American Autonomic Society for the most outstanding abstract. Today, he is finishing his residency in Rehabilitation and is involved in several studies at the department of Physiology at the Radboud University Nijmegen Medical Centre.

LIST OF PUBLICATIONS

Groothuis JT, Thijssen DH, Lenders JW, Deinum J, Hopman MT. Leg vasoconstriction during head-up tilt in patients with autonomic failure is not abolished. J Appl Physiol, 2010 Dec 2. (Epub ahead of print). *(Chapter 8)*

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